CHAPTER-VII

DISCUSSION
DISCUSSION

(1). **Physico chemical analysis of polluted and unpolluted soil samples**

The moisture content of the soil was found to be more in ammonium hydroxide polluted soil followed by cement dust polluted soil, unpolluted garden and agriculture farm soil and sewage polluted soil, unpolluted garden and agriculture farm soil. The size and shape of soil particle and aggregates have a profound effect on the moisture content may be attributed to the difference in soil texture. Soil texture refers to the relative proportion of the various groups of individual particle. The electrical conductivity of the soil solution gives an idea of the total soluble salts of the soil.

The soil has the ability to assimilate certain amount of waste products but the regular flows of industrial effluents in large quantities in fields pollute the soil. When the soil physico-chemical properties are altered by the pollutants the organisms suited to the changed condition survive whereas the susceptible ones disappear or become restricted. Land application of sludge or compost can cause beneficial as well as harmful effects. Microorganism is present in all soil. In fact even in the presence of high amount of toxic substance complete destruction of the microflora is possible. The physical characteristics of the soil are equally important as biological and chemical characteristic for pollutant attenuation (Kore et al. 1976, Fuller 1977, Fuller 1978, Fuller et al. 1981, Cope et al. 1983). Some prominent physical soil characteristics and processes that influence
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movement and retention of pollutant in soils are (a) texture, (b) surface area, (c) soil structure, (d) stratification and materials, (e) compaction, and drying and wetting. All of the factors that influence soil permeability play an important role in pollutant attenuation by soil. Relationships between physical, chemical and biological processes are diversely interrelated and difficult to identify (Fuller 1978, Lindsay 1979 Jenny 1980). Soil structures describe the arrangement of soil particle into larger units. Thus, soil structure can control the rate of fluid flow through soil, water holding capacity and solute retention. The non industrial sludge contains fewer heavy metals than the industrially influenced sludge. The quality of raw sludge is emphasized because like all other residues and water, the loading rates and capability of maintaining soil productivity depend largely on these characteristics. Raw sludge usually contains large proportion of soil particles. Sludge consist of organic carbon (organic matter), nitrogen, phosphorus sulfur and heavy metals like Cd, Cr, Cu, Hg, Ni, Pb and Zn. The lands most suitable for waste utilization are those having only slight slopes and moderate soil texture. Lands suitable for solid wastes are not necessarily suitable for waste water. Soil irrigated with effluent discharge shows marked visual effect in soil colour. The higher concentration of these chemicals in effluents shows adverse effect on normal physico chemical properties of the soil such as porosity, pH, total nitrogen, exchangeable cation such as sodium and potassium. Me Calla (1942) and Johnson (1957) attributed high infiltration rates of the soil of changes in soil physical properties resulting form organic matter decomposition.
Ammonium hydroxide polluted soil showed high sodium nitrate nitrogen and ammonia content as compared to raw sewage and cement dust polluted soil samples and unpolluted garden and agriculture farm soils. Maximum potassium content was recorded in case of cement dust polluted soil and minimum in case of ammonium hydroxide polluted soil is induced by high sodium content of soil (Black 1968). The porosity of the soil decreases as the depth increases. The porosity of soil was found to be high in case of raw sewage polluted soil, followed by unpolluted garden and agriculture farm soil, ammonium hydroxide and cement dust polluted soil. Porosity decreases as the sodium content of the soil increases. Kelley (1963), Black (1968) and Hesse (1970) have also pointed out that high sodium content adversely affects the physical properties of the soil, decreases permeability and clay particle fill up the pore space of the soil. Bulk density decreases as the porosity increases.

The relative increase in organic carbon and decreases in nitrogen was found to be in cement dust polluted soil as compared to other polluted and unpolluted soils which could be due to retarded microbial activity in soil resulting less release of nitrogen through decomposition of organic matter. The pH value of cement dust was more than the raw sewage and ammonium hydroxide polluted soil, unpolluted garden and agriculture farm soils. The increase in pH could be due to accumulation of calcium and aluminum hydroxide formed during hydration of cement dust (Pajenkemp 1961, Gzaja 1962).

The organic matter was determined more in cement dust polluted soil followed by raw sewage polluted soil, unpolluted garden soil and agriculture
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farm soil and ammonium hydroxide polluted soil. The possible reason for high content of organic matter in cement dust polluted soil may be due to reduced microbial activity and consequently low rate of the decomposition for gradual accumulation of organic matter. The organic matter of sludge may improve the soil physico chemical properties (Bengtson and Comette 1963), Epstein 1974).

Raw sludge, however, often contains undesirable chemicals which may be toxic to plants as well as to soil microbes. Movement of inorganic phosphorus through soil is extremely limited in almost any soil. Soil texture and soil depth influence retention or renovation of phosphorus more than any other factor. The cement dust forms a crust on soil surface. It is already known the crust upsets the growth and development of plants (Peirce 1910, Czaja 1962) influencing, therefore, the microbial population.

(2). Morphological studies of test pathogen

Morphological character of 10 isolates of the fungus isolated from the samples collected from different parts of the country were studied difference were observed in cultural appearance like, colour growth rate period of initiation of sclerotia and conidia. All the 10 isolates divided into 5 groups on the basis of their colony character. Isolates CO₁ produced light brown scanty mycelium. Isolates CO₅, CO₇ have light brown, aerials, mycelium where as isolates CO₂, CO₃, CO₉ and CO₁₀ light brown, effuse growth. Only three isolates Viz.-- CO₄, CO₆ and CO₈ produced dark brown fluffy growth. Difference in cultural character of these fungus have, been observed by Tullis (1933 b). All the 10 isolates of the fungus produced sclerotia and varied in the initiation period in scleratial formation and its size. Isolates
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CO₃ produced sclerotial initials within 96 h, isolates CO₁, CO₆, CO₉ and CO₁₀ after 120 h, isolates CO₅ and CO₈ after 132 h, and isolates CO₂, CO₄, CO₇ after 144 hrs. of incubation period. Mundkur (1935) and Punter et al. (1984) have reported the sclerotia of the fungus were usually produced after a week but in some isolates it may develop after 10 days. Ahuja et al. (1987) have, also reported similar observation on the sclerotial formation. The sclerotia produce in culture were bigger in size than the sclerotia produce in nature. This may be due to the availability of better nutrition in the medium than on its natural host. Similar observations have been made by Ahuja et al. (1987). The ten isolates of fungus shown variation in the size of their sclerotia and have been divided into two groups. In the first group the isolates had sclerotia smaller than 250µm and the second group more than 250µm average diameter. Park and Bertus (1932) reported 175 180µm diameters of the sclerotia where as Tullis (1933 b) reported that the sclerotial size 180 280µm with an average of 230 270µm.

Ferreira and Webster (1975 b) found no relationship between production of conidia, sclerotia and growth rate with virulence in evaluation 20 isolates of the pathogen. In the present investigation no direct correlation could be established between the sclerotial conidia and hyphal diameter of 10 isolates collected from different part of India. This indicated that it is not the diameter of the individual hypha and but the type and number of the hyphal aggregation which is a function of sclerotia formation and plays an active role in the process of disease development. Although S.oryzae has a peculiar nature to form sclerotia and conidia in the same generation in the same season so of the isolates Viz. Raipur, Panlnagar, Sonbhdra and CRR1...
(Orrisa) were not produce the conidia during the establishment of disease. Although the exact mechanism of non formation of conidia by these isolates is not understood. In the soil born sclerotia fungi sclerotium formation involve partial inhibition of mycelial growth, external and internal morphogenetic factors (Chet and Henis 1975). It is probable that S.oryzae may carry the genetic information of sclerotium and conidium formation. Yet only under suitable external condition which in turn effect, the internal composition of the cell this genetic information is translated into metabolic and morph genetic events. In the present investigation out of all the isolates the Bhadohi (U.P.) isolates having the largest sclerotia (255µ) and largest conidia (72×14µ) was not the most virulent but the Panlnagar isolates with smaller sclerotia (210µ) and no conidia. As the most virulent so on the basis of present investigation it is clearly indicate that the disease development does not depend on the sclerotal size but depends on the energy potentiality of the sclerotal propagules to infect the rice host.

(3). Physiological studies of test pathogen

(a). Studies of pathogenicity

On the basis of virulence of 10 isolates of S.oryzae tested on 12 varieties under pot and field condition have divided into various group. It was observed that not only was there a wide range of variation in the virulence of the ten isolates of the pathogen S.oryzae (catt.). Under study but also there was a differential reaction of the rice varieties of the isolates. Ahuja et al (1987) reported that Jaya was susceptible against four isolates of S. oryzae while it have been reported resistant/tolerant to the fungus (Srivastva et al 1971, Amin–1976). But in the present study it was found to
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be resistant to isolates CO$_3$ and mostly very susceptible to remaining isolates. The variety prasad exhibited resistant reaction against all the isolates except the CO$_1$ and CO$_2$ where it was moderately resistant. This finding was also supported by observation of Ahuja et al. (1981). There is no information available on the reaction of varieties Ratana and Saket against these disease but in the present investigation Ratna and Saket was found to be resistant nearly all the ten isolates. Basmati and Govind exhibited similar disease reaction to all the isolates, was found to be either moderately or very susceptible but not resistant. Indrason and IR 36 exhibited some disease reaction to all the isolates except against isolates CO$_{10}$, which gave resistant reaction to IR 36. The variety Manahar gave a resistant reaction except against isolates CO$_3$, CO$_7$ and CO$_{10}$, which give moderately susceptible and isolates CO$_6$, which gives very susceptible reaction. IR 50 is similar to IR 36 except against isolates CO$_3$ and CO$_8$ it was found to be resistant. The prominent variety Manshuri was susceptible to all the isolates except against CO$_1$, CO$_8$ it was found to be resistant. The present study showed that there was considerable variation in the pathogenicity of the different isolates of *S.oryzae*. It is therefore of at most important that much care should be exercised in the selection of *S.oryzae* isolates and several representative isolates be used when screening rice variety against stem rot.

In view of the evidence of differential reaction of rice varieties with the isolates of *S.oryzae* intensive studies are called for to standardize the condition of artificial in injection to select out a suitable set of differential and to determine the number and distribution of such pathogenic strain in the country. So that breeding for resistance to stem rot could be planned on the
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basis of a full and detailed knowledge of specialization in pathogenicity in
*S. oryzae*. Thus the result indicated that variation in virulence, exist in the
different isolates of the pathogen and there is presence of different degree of
resistance in the rice cultivars against the disease. In general local isolates
were less in virulents as compared to the outer isolates.

(b). **Nutritional studies of the pathogen**

**Nitrogen**

Nutritional study of the fungi with nitrogen had been studies by
Conchrane (1958), Foster (1949) and Lilly and Barnett (1951). It is used by
fungi in the form of nitrate, ammonium, organic or elemental nitrogen both
for functional as well as structural purposes. Nitrate have been reported to be
an excellent sources of nitrogen for imperfect fungi and Ascomycetes
(Agarwal & Gangulli 1960, Bilgrami 1964, Subramanian and Tyagi 1968)
etc. The capacity to use nitrate depend upon the nitrate reductase activity in
fungi. In the present study ample vegetative growth of both mycelia and
sclerotia of *S. oryzae* was seen ammonium nitrates as a source of nitrogen. A
high concentration of ammonium is toxic and this toxicity is influenced by
many factors such as the presence of carbon sources amino acid phosphates
etc. Thus this finding is in contrast to report on similar nitrates utilizing
fungi, which are normally unable to utilize inorganic ammonium including
ammonia nitrate (Brain et. al. 1947, Morton and Macmillians 1954 Apparao
1956). It is possible that in the metabolism of *S. oryzae* nitrates are not
reduced to ammonia but enter the metabolic cycle at a higher oxidation level
probably hydroxiamine and take the oxime pathway thereafter (Silver an
MceIlroy 1954). The improved growth of *S. oryzae* in weakly ionized
ammonium salt and ammonium nitrate due to the beneficial effects of organic acid indicates that these organic acids act primarily through aiding assimilation of NH$_3$ ions probably in the formation of amino acid and in the metabolic pathways. This implies that during the course of application of nitrogen by the rice plants providing a congenial substrate both in the soil and plant for nutrition of _S. oryzae_ and leading to severe disease incidence. In fact there is report that in field trial increased application.

**Phosphorus**

Phosphorus is utilized in the form of phosphates both as a structural and functional non metallic element in the growth of fungi. It forms the basic component of nucleic acid (DNA, RNA) the high energy, rich phosphates (ATP, ADP) and the phospholipids. Some of the fungi like _achlya sp. Isoachlya. Anisospora and Saprolegnia_ sp. (Bhargava 1945) and _Fusarium Solana, Botryodiplodia Ananassae, and Macrophomina phasalina_ (Bhargava and Tondon, 1963) do not grow at all in the absence of P. Some fungi like _Curularia_ sp. (Agarwal 1953) and _Alternaria tenuis_ (Singh and Tondon 1967 ab.) are at least able to grow feebly on P, deficient media. Thus fungi exhibit varying requirements of P, in the present study also _S. oryzae_ grew well known in NaH$_2$PO$_4$ amended media in lower concentration up to 8 mg/l, nutrient solution, but at higher concentration (9 10 mg/l) P, was inhibitory to the growth of the fungus probably due to toxicity. Since P, is utilized by _S.oryzae_ in its nutrient substrate so the probability of increased stem rot on rice plants receiving moderate P is not obscure but the same is not true with higher P application. Supply of low P generally suppresses the early N uptake and prevents protein synthesis.
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resulting in accumulation of Non protein N. However plentiful supply of P as has been advocated in recent year to the rice growers for making green revolution as success not only accelerates the seedling growth and increase the content of P in form of nucleic acid and phospholipid (Fujiwara 1965) but also may indirectly control the incidence of stem rot disease of rice.

**Magnesium**

Magnesium is an element of the alkaline earth group and essential for green plants and animals as well as for fungi and bacteria. Many enzymes system are activated by magnesium ions. Magnesium is involved in many of the enzymatic reaction in fermentation (Summer and Sommers 1947). The nutritional effects of magnesium have been investigated in cause of number of fungi. *Penicillium glaucum, Botrytis cinerea and Alternaria tenuis* fail to grow in the absence of magnesium (Rabinovitz, Serana 1933). The magnesium nutrition of *S. oryzae* grew better in MgSO₄ amended medium up to a concentration of 6 mg/l nutrient solution but at higher concentration it grow declined probably due to elemental toxicity. Since mg has an important role of N. metabolism and Si uptake the plant deficient in mg are easily attacked by fungal pathogen (Tankara 1957). More so in combination with Ca it forms the pectate, which constitutes the structural component of middle lamella of plant tissues. It is inferred from the present study that a higher level of mg content in rice plants or in soil is determined to the growth of *S. oryzae* and induce resistant to stem rot disease.

Though the exact amount of mg in soil solution is not available its slow release in natural soil may help the growth of *S. oryzae*, which is evident from the present study. Further the mixed mg in mineral such as
magnesium (MgCO₃), Olvinine (MgFe)₂SiO₄ growth of this fungus in the soil. In fact dolomite (MgCO₃, CaCO₃) may also effects the growth of this fungus in the soil. In fact dolomites and its products are the most popular and economies sources of magnesium fertilizer (Harra 1959) which when added to the soil may influence the occurrence of pathogen inciting the stem rot disease.

**Potassium**

Among all the metallic minerals K is present in the largest amount in the both mycelium and pores concentration of 0.001 and 0.004 M of this metal are adequate for most of the fungi (Steinberg, 1946, Jarvis and Johnson, 1950). The essentiality and physiological role of potassium in fungal metabolism has been little investigated. The potassium nutrition of the stem rot pathogen *S.oryzae* has not been investigated so far. In the present experiments *S.oryzae* was allowed to grow in nutrient medium ameliorated with KCl and growth was found proportion to the concentration up to 5 mg/l, beyond this concentration there was abrupt decline in growth of the fungus. This indicates that the fungus can, not utilize higher concentration of K, which may be inhibitory to its growth. This implies that heavy application of potash fertilizers in the rice plants may be reduce the incidence of stem rot disease in the growing seasons. This consideration is in agreement with the finding of (Jain 1978). Earlier reports indicate that K is absorbed vigorously in the early stage of growth (Fujiwara 1965) and the organic material is also known to contribute to increase the available of plant nutrient. (Oh 1978). These conditions may lead to the retardation of the stem rot fungus.
Sulphur (S)

It is now well known that S plays a significant role in the metabolic activity of fungus. This is essential for the biosynthesis of sulphur containing amino acid besides being a component of SH or thioles group of many enzymes (Bhargav and Tondon 1963, Cachrane 1958, Lilly and Barnnet 1951) co enzymes and vitamins which in turn effect various vital process in fungi including their choice and ability to utilizes various source of Sulphur.

The fungi like *Pythium* spp. (Saksena et.al. 1953) and *Pestalotia malorum* (Tandon 1950) are able to utilize a variety of S sources. The sulphur nutrition of *S.oryzae* however is not known in literature. The growth response of this organism to sulphur was seen in BaSO₄ amended medium up to a concentration of 6mg/l nutrient solution. However in all the concentration under study the growth trend of *S.oryzae* is quite less in response to sulphur than in nitrogen, Phosphorus and magnesium. Since *S.oryzae* has the ability to utilize sulphate sulphur it can be rightly called as enthio trophic fungus according to Volkonsky (1934) terminology. It need further investigation as to whether this organism can utilize organic are elemental sulphur or behaves as parathiotrophic by utilizing reduced sulphur for its growth purposes. Soil S is found primarily in the organic fraction (Quastel 1963) but may also be found in mineral as pyrite cobalts gypsum and apsomite and in the soil solution as the sulphate ions (SO₄²⁻) which may be dispensable to the soil born pathogen *S. oryzae* for nutrition and subsequent growth.
Molybdenum

Molybdenum is known to be required for the formation and activation of nitrate reductase in fungi. (Nicholas and Nason 1954 a) showed that the nitrate reductase enzyme for *Nurospora crassa* is a flavoprotein which contain Mo. In the modern agricultural technology the spraying of Mo in the form of ammonium molybadate at a very low concentration has become a blanket recommendation for the all crop plants for increasing the protein content. Since the essentially of Mo was first discovered by (Stainberg 1936 b) its nutritional effect on *S.oryzae* has not yet been studied by any worker. In the present study in a nutrient medium containing \((NH_4)_6M_6O_{24}\), the maximum growth of the fungus occurred up to a concentration of 2 ppm/l and the growth declined abruptly at higher concentration probably **NH**\(_4\) interfered in the utilization of Mo by *S.oryzae* at higher concentration as happen in cause of *Aspergillus niger*. Where molybedonum requirements are higher of nitrate and not **NH**\(_4\) is used as the nitrogen source (Stainberge 1936) since there is a decreased growth rate of *S.oryzae* in response to increased Mo supplement in the basal nutrient medium. The incidence of stem rot disease can be correlated with the quantitative occurrence of Mo either in the soil substrate or rice host tissue. It will not be irrelevant to disease in this content that probably due organic amteiorates in the soil Mo availability increased as both the factor are directly associates organic amended soil (Kubota et.al 1961). Similar results, was also obtained by Gupta (1971), when Mo was supplied in the high humus soil.

Although, rate of Mo as phytoalexin inducer has been elaborated (Sinha and Giri 1979) a lower degree of induced resistance under its
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influences in comparison to B and Mn in the nutrient solution probably might be an effect of the pH of the medium because its solubility decreases with decreases in pH (Ishizuka 1971). Though Mo requirement for rice plant is as such very low it may be considered as part and parcel in the summation of micronutrients in the plant develop resistance against the stem rot disease incited by *S. oryzae*.

**Copper (Cu)**

Copper is present in many enzymes as tyrosine and it may also play a role in the chlorine metabolism of fungi. The growth of some fungi like, *Aspergillus niger*, *Fusarium oxysporum*, *Cercospora nicotianae*, *Sclerotium rolfsi*, *Theilaviopsis basicola* and *pythium irregulare* was reduced due to lack of Cu in the media (Steinbery. 1936 a, 1950). Saraswati Devi (1958) however could not establish the indispensability of Cu for the growth of some soil *Fusaria* studies by her. But in the present study of soil born fungus *S. oryzae*. It is found that it can utilize Cu in a CuSO₄ amended medium at lower concentration up to 2ppm/L. like molybdenum. At higher concentration of this Cu compound the fungus can, not grow well probably due to toxicity. Though the Cu is antagonistic to *S. oryzae*, it attracts carefully, attention in its application to the rice plant in controlling the stem rote, disease. Since the critical phytotoxic effect of this element on rice plants have been observed to be at 30 ppm (Yoshida et.al 1976) and 10⁻¹⁰ m concentrations (Sinna and Giri 1976). Very little cu is found dissolved in the soil solution (Wikalander 1957) estimated that the soil solution of ordinary soils contains 0.01 ppm Cu and the actual water soluble amount
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does not exceed 1% of the soil. The occurrence of appreciable amount of Cu in the soil may exert detrimental effect on the growth of *S. oryzae*.

**Silicon (Si)**

Silicon is the most abundantly available elements on the earth's crust. The stem rot, fungus *S. oryzae*, being a soil born perthotroph is subjected to the affluence of this element every now and then in its habitat. Further the rice plant being typically, silicicolous has been observed by (Kono and Talhandi 1958) to absorb silica at fantastic rate against the concentration gradient and during vigorous growth a single plants could absorb 0.1g Si in 24 hr. However the rice plants under Indian condition are known to contain lower amounts of silica (Tankaand Yoshida 1970, Pantnaik 1971) probably become the available Si content of a majority of rice growing soil is reported below the critical limit (Takaya and Kyuna, 1968). In deep water, cultivation there is more acceleration of Si movement into the plant at the reduction zone. That the flooding increases the availability of silicon in soil has been demonstration by many workers (Ponnamperuma 1965). Thus due to the presence of Si in and around the rice plant was thought pertinent to study the growth trend *S. oryzae*, with special attention to Si in the present investigation in the Na$_2$SiO$_3$ amended nutrient medium. It is found that silicon has less nutritive values for this organism that copper and molybdenum in all its concentration. The maximum growth of the fungus is found only at a concentration of 1 ppm/l. nutrient solution and this growth start decreasing at higher concentration. These indicate that *S. oryzae* extends least performance for silicon like other microelements rather than growth of this fungus is seriously inhibited at increased level of this element. Thus
silicon as a major constituent of soil should not be misconstrued to play a role in the virulence of widespread occurrence of this soil-born pathogen in rice soils rather it may contribute to resistance. Although, (Sridhar and Mahadevan (1979) have expressed doubt about the role of silica in the disease resistant their viewpoint was not substantiated with experimental data. That the presence of excess Si in plant tissue increases resistant to fungus diseases through mechanical hindrance of infection peg is also not new (Yoshii 1936). In the present investigation, since there is a negative growth trend in response to increased level of Si in the nutrient medium, excess Si in the rice plants is a factor for resistance. In the mechanism of host parasite interaction of one believes Cobbs mechanical theory then Si must have its role which can be increased to induce resistance in the rice plants.

**Boron (B)**

The application of B plays a role in the resistance of brown spot disease in rice (Akai 1955), club root disease in cabbage (Antonova et al 1974) and yellow rust disease in wheat (Ismailow 1954). In the present study when *S. oryzae* was subjected to Na$_2$B$_4$O$_7$ in the nutrient medium it was observed that its maximum growth takes place at very low concentration (1 ppm/l.) and it declined abruptly at all other higher concentration. The growth of the fungus is very negligible in response to B compared to all other elements in the present study. It appears that B can be best utilized to minimize or control the stem rot disease of rice. Boron appears in exchangeable soluble and non-exchangeable forms in the soil that is as boric acid (H$_3$Bo$_4$) calcium or magnesium borates and as constituent of silicates (Bould 1963, Wiklander 1958). Like Zn the dissolved B content in the soil
solution is very low. Analysis of different soil have indicated that the amount of B in organic soils may be higher than those found in acid soils of humid regions where B deficiency is likely to occur. So during the course of organic amelioration by the rice growers the B accumulation in the field may inadvertently control the stem rot disease. Close involvement of B in the soil wall differentiation is probably determinative factors which form a polyhydroxy complex in plants in the form of various carbohydrates and pectic materials (Epstane 1972, Spur 1957). The B deficient condition thus not only decreased the synthetic process in rice plant but also probably causes immobilization of sugar decreasing its level in the peripheral tissue of the stem thereby accounting for its greater susceptibility to the stem, rot disease.

**Nickel and Tin**

The specific roles of these two elements in the physiology and metabolism of fungi including *S.oryzae* have not been investigated thoroughly so far. The fungal nutrition of these, microelement is probably doubtful. Though (Richards and Troutman 1940) found B and Sn in the yeast ash by spectrographic analysis during the investigation of its composition the mere presence of these elements in fungus cell does not necessarily mean that these are essential nevertheless since Ni and Sn occurs in tracers in the soil profile and *S.oryzae* is a typical soil born rice pathogen showing wide variability in its nutrition habit. It was through pertinent to study the effect of the above elements on these pathogens. The result indicate that *S.oryzae* can be utilized Ni and Sn for this nutritional purpose only at a concentration of 1 ppm/l. nutrient solution and this growth is very
insignificant compared to other elements under the present study. The nutrition of these unusual micro organisms in traces by *S. oryzae*. In the isolated medium may brobably be due to production of adaptive enzymes do novo (Lilly and Barnett 1951). At higher concentration (2 10 ppm/l.) the fungus exhibits very negligible growth indicating that Ni and Sn appears to inhibits the stem rot fungus to an appreciable extent, since there is a negative correlation between the concentration of Ni and Sn and the growth trend of *S. oryzae*. It is suggested that the soil application of organic amendment for chemical compound containing these element may significantly reduced the stem rot disease of rice incisted by this pathogen.

**Iron**

Iron seems to occupy an intermediate position between the macro and micro essential elements. It does not enter into the composition of the food or the composition of the fungus itself. Iron is an activators constituent of many enzymes such as peroxidases, catalases and cytochromes. Iron effects the nutrition of good number of fungi, *Glucosporium psidii* and *G. eitricolom*, required 25Hg/L concentration of iron to attain maximum growth as well as good sporulation, while third species *G.limettieolom* needs a steel higher concentration of iron i.e. 50Hg/L. Although its sporulation is best only at 5 Hg/L. concentration (Tandon and Agarwal 1956). *Alternaria tenuis* attains best growth at 250Hg/L, concentration of iron (Grewel 1956). Iron is also very essential for *Fusarium* (Saraswati Devi 1958) and another rice pathogen *Pyricularia oryzae* (Apparao et al 1955, Apparao 1959) also should that iron is an effective antagonist against certain toxic ions such as Cu and Zn during studies with *P. oryzae*, In the present study the optimum
growth of *S.oryzae* in response to FeCl₃ amended medium occurred at a concentration of 4 and 5 ppm/L. At higher concentration its growth decreased slowly. The exact mechanism of effect of irons has not yet been cited in literature. Soils are generally not deficient in ions. Appreciable quantities of iron are present in minerals inhydrate oxidies such as limonites (Fe₂O₃.H₂O) and in the sulphide from (Bould 1963). The slight presence of iron whether ferrous or ferric in the soil may help better growth of the soil inhabiting pathogen *S.oryzae* as evident from the present study.

**Calcium**

It has been established that Ca is very essential for a number of fungi (Cochrane 1958) and Ca is used variously by the fungi it acts as a protective against the injurious effect of certain common monovalent cation specially H⁺, Na⁺ and K⁺. It has also been found that Ca effects the time required for perithecial formation of *Cheatomium* (Basu 1951). The intensity of condition in *Trichoderma viridis* is also effect by Ca concentration (Brain and Hemming 1950) although the exact function of calcium is not known for *S.oryzae*. It influences the nutrition and is essential for its growth the optimum growth of *S.oryzae* occurred in CaCl₂ amended medium at a concentration of 5 ppm/L. At higher concentration Ca, is less utilized by *S.oryzae*, for nutrition than ions. Generally Ca is the major exchangeable cation of the fertile soils. (Marshall 1951). However the major portion of Ca in the soil is found in a non exchangeable form chemically bound in primary minerals such as anorthite (CaAl₂ Si₂O₈) or calcite (CaCO₃) in semi arid and arid regions and through weathering it is made available to the plant and soil
born biological entities. Since S. oryzae responds to the nutrition of Ca, it can utilize it in the soil for its growth purposes and increases the biomass.

Zinc (Zn)

Zinc is an activator component of most of the enzymes such as carbonic anhydrase, alcohol dehydrogenase, lactic dehydrogenase, glutamic dehydrogenase etc. Zinc has also been shown to be a structural compound for ribosomes. Even high level of zinc induces genetically stable variants in Helminthosporium sativum (Millikans 1940) though the effect of Zn on the physiology and metabolism of S. oryzae has not been investigated so far it has been established by this experiment that Zn is utilized for the growth of this fungus. The optimum growth of S. oryzae occurred in ZnSO₄ treated medium at a concentration of 4.5 ppm/L. nutrient solutions from the nutrition point view Zn was less effective for the growth of S. oryzae than even Fe and Ca. (Bould 1963) reported that Zn occur in the ferro magnesium mineards, magnetite, and biotite and horn balaned. Weathering of these minearals releases Zn in the divalent from which is readially absorbed into soil and organic matter in exchangeable form. Although little is known about the concentration of Zn in the soil solution, it is generally thought to be quiet, favorable for the growth of S. oryzae according to the present finding.

Manganese (Mn)

It is very essential for the growth of Aspergillus niger (Donald et al. 1952 Sulochana and lakshmann 1968) in S. oryzae, however the magnese
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Nutrition has not been studied so far. The optimum growth of this fungus is observed at 12 ppm/L in MnCl₂ amended medium. These indicate that *S. oryzae* is able to utilize Mn in traces for its growth purpose. At higher concentration elements is inhibitory to the growth of the fungus and result in a sharp decline of mycelia mat. In the maximum stages Mn is superior to Zn for the nutrition of *S. oryzae*. Besides its nutritional values, Mn may have the same effect on physiology or metabolism of the fungus, which needs further investigation. The organic ameliorates provides the facilities to release of nutrient including Fe and Mn to the soil solution (On 1978, Yoon et al. 1975). According to Deeper (1947) the Mn of the soil may exist in the bivalent, trivalent and/or tetravalent form. The bivalent ions may be found dissolve in the soil solution or as an exchangeable ion absorbed to the soil born rice pathogen *S. oryzae* inhibiting its growth at higher concentration as was observed in the present study.

**Cobalt (Co)**

Although the evidence indicating that the essentiality of cobalt has been presented quite often (Marston 1952) it is doubtful whether it is needed for fungal growth. In bacteria, actinomycetes and animals Co is known to be essential for the synthesis of compounds of the vitamin B₁₂ group (Cobalamin). Cobalt forms a constituent part of the tetrapyrole ring of this vitamins. Fungi have now been shown to synthesize cabalamins (Tunner 1960). Some fungi are reported to be deficient in cobalamin. (Adair and Vishniae 1958, Litman and Miwatani 1963) pertinent literatures are not available on the effect of cobalt on *S. oryzae*. The maximum growth of this
fungus is seen at a concentration of 1 ppm/L in the test medium containing CoCl₂. At higher concentration of Co, the growth of the fungus was reduced like that of Mn, Ni, Sn, Zn and B. Whether Co is essential for the growth of S. oryzae or it has the ability to alter the physiology of this organism is a matter of conjecture and needs intensive research.

(c). Study of parasite relationship in varying region of environment conditions.

(i). Effect of temperature

Among the various natural factors, temperature play an important role in geographical distribution of fungi (Bisby 1943). The specificity of temperature requirement may be for the maximum activity of enzymatic system favorable for growth. The production and germination of the spores favorably effected of the temperature.

Maximum average growth of the isolates when grown on PDA was observed at 30°C followed by 25, 20, 35 and 15°C while no growth occurred at 40°C. The optimum temperature of 30°C was found for all the isolates except isolates CO₄ and CO₅, which had an optimum temperature of 25°C for maximum growth. The optimum temperatures of 30°C for the growth of the fungus have been reported by several workers (Hemmi and Yobogi 1927, Tullis & Crelly 1933, Sprague 1950). Isolates CO₄ and CO₅ which grew better at 25°C may be due to their adoption to the localities as these were isolated from Raipur and pantnagar respectively. Raipur and Pantnagar is hilly areas where night temperature becomes lower as compared to the
plain, so it appear that these isolates were adopted them self to little lower temperature.

(ii). Effect of pH

On the basis of present study the radial growth of the isolates occurred, within a pH range of 4.0 to 8.0 but the maximum radial growth of the all isolates occurred at pH 6.0 followed by 6.5, 5.5, 7.0, 5.0 7.5, 4.5, 4.0 and 8.0. These observations support the work of earlier worker (Anonymous 1930, Tulis and Cralley 1933, Ono & Suzuki 1960, Sethunathan 1963 & 1969). The effect of different pH on individual isolates did not show any difference, pH 6.0 was observed to be optimum for all the isolates indicating that where is no difference in biological activity of isolates in relation to pH. Though difference in cultural and morphological character like colony diameter and its colour period of intition in sclerotial formation and its size and interaction to media, temperature and pH to different isolates were observed these are of minor significance to be used for there grouping.

(d). Mode of infection and disease development

On the basis of histopathological study it has been observed that sclerotia produced germ tube, formed a mycelium on the surface of the host just after the aggregation. The mycelial mat form an infection cushion/appreseoria from which multiple pentration of cuticle occurred through infection pegs. The infection process of the pathogen has not been studied thoroughly. (Ono 1953) reported that prior to infection, the pathogen produced appressoria and infection cushion. It is well known that cuticle is composed of wax and cutin and cell wall of epidermis is made up of
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cellulose, pectins and hemicellulose. The pathogens grow on the surface of host to achieve mechanical and chemical strength to the defense barriers of the host before penetration by producing sufficient quality of necessary enzymes. In *S. oryzae* possible involvement of cell wall degrading enzyme like pectolytic and cellulolytic have been indicated some fungi posses the necessary enzymes for cuticle, digestion (Heinen & Linskens 1960) and there is same indication that fungi are capable of producing the necessary enzymes for host cell wall digestion (Albersheim 1969).

(e). **Survival of the pathogen in adverse condition of temperature and depth**

(i). **Survival of the fungus in different temperature**

Knowledge of the mode of survival of a pathogen during the off seasons is one of the important components that help in the management of the disease. The results on survival of the pathogen showed that its sclerotia survived up to 17 months at 10°C, 16 months at 20°C and at room temperature 15 months at 30°C, 14 months at 15°C, 12 months at 25°C and up to 6 month at 35°C. Sclerotia of the fungus have been reported to remain viable for 3 years at 20°C, 10 13 months at 25°C, 4 months at 35°C and 12 months at 35°C, when submerged in top water (Nishikado and Hirata 1937). The sclerotia remain viable up to ten months (Mishra ed.at. 1966) and 12 months (Kim and Kim. 1988) at room temperature. There is no report in literature on the survival of the sclerotia at the corresponding temperature studies presently but the survival at 20°C in Japan has been reported to be
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much longer than the period observed in the present study present finding were also supported by earlier reporter.

(ii). **Survival of the fungus in different depth:**

The experimental finding reveal that sclerotia of the fungus at different depth in soil remained viable up to 11 month at 5 cm, 10 month at 10 cm, 9 month at the surface and 6 month at 15 cm depth in soil. When straw containing sclerotia was buried 10-15 cm deep in soil in Arkansas a small percentage of viable sclerotia were recovered after 6 years. In the present observation it is not certain whether all the viable sclerotia reported were original ones added to the soil are newly formed due to some growth of the fungus. In another study when sclerotia were placed at a depth of 5, 15 and 25 cm and at the surface of the soil it was found that the viability of sclerotia decreased more at the surface soil than other depth (Usmani and Ghaffar 1981). But in the present study the maximum period of viability were observed at a depth of 5 cm and it decreased as depth increase. Sclerotia of the fungus remained viable for 133 days when buried in moist paddy soil (Park and Bertus 1932).

(4). **SOIL AMENDMENTS IN RELATION TO THE DISEASE**

(a). **Application of chemical fertilizers N.P. and K.**

The present investigations indicate that heavy application of nitrogenous fertilizers resulted in higher incidence of stem rot. Similar results were obtained by Rayes (1929) in the Philipines, Nikata and Kawamura (1939) in Japan, Crelly (1939) and Templeton (1962) in U.S.A. Pracer and Luthara (1944) and Anonymous (1958) in our country. The result further indicated that application of P in low doses (60 ppm) was found to increases the incidence of disease. This finding also provided by result
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obtained by Cralley (1939) in both glass house and field experiments. Application of higher levels of P (120 and 180 ppm) in combination with N was found to reduce the disease, that results are in agreement with those obtained earlier (Anon 1958) in the manorial experiments conducted at Aduthuray (Tamilanandu) that high level of P had an ameliorative effect.

In the present investigation the application of K in high doses (120 and 180 ppm) in combination with N was found to reduce the disease incidence. The beneficial effect of K in reducing the disease also been reported by several workers (Adair and Crely 1950, Okamoto 1950, Templeton 1962) but according to Keim (1972) increasing amount of P and K level in rice leaves had no significant effect on stem rot development. Further it is noteworthy that at an excessively high dose of N (240 ppm) with no application of P the disease incidence remained relatively low irrespective of the level of applied K. But it has been seen that under high yielding variety program fertilizes of rice field with heavy doses of nitrogenous fertilizes is considered essential for maximum production of most formers. The result presented here indicated that stem rot in an infected field can be expressed to increase with increasing rate of N up to 160 ppm and of P up 60 to ppm. It is therefore important to minimize infection of the crop by reducing the supply of N to a low level.

(b). Application of green & organing manuring

It has been emphasized that green and organic manuring greatly influenced that percentage infection of different concentration. As the doses increase the percentage of infection decrease at the increasing the days of inoculation. The soil applications of green and organic manures for the
control of various soil born diseases have been reported by various workers. King and Loomis (1926) studied the effect of organic manure on the control of root rot (*P. omnium*) of cotton and observed a consistent reduction in the incidences of the disease in infected areas. Control of the disease through application of organic manure, was also reported by King and Hope (1934). Kling further observed that 30 tons of green manure applied per acre reduced to 1.6 percents as compared to 56.2 percent in untreated plots. Manzies (1962) observed that micro sclerotia of *V. dehlie* survived almost in normal soil but were killed in 5 days when such soil was amended with 1 percent algal meal several other examples of effectiveness of green manuring to control rot disease including root. Rot of straw berry with soyabean residue (Hildebrand and West 1941). West and Hilbrand (1941) sclerotia on potato seab with incorporation of soyabean cover crop in the soil.

Several other workers have also emphasized upon the importance of green and organic manuring upon fungal disease. Kulkarni and Kulkarni (1995) have investigated the effect of organic amendments and green manuring on the survival of color rot fungus (*S. rolgsii*) of groundnuts. Davis et al (1996) have reported the effect of green manures on verticillium wilt of potato.

(c). Effect of soil moisture at various levels

On the basis of observation it has been seen that moisture has great influences on the percent infection of rot disease of rice caused by *S. oryzae*. Coach H.B and D.W Henderson (1967) studied the application of soil moisture principal to plant disease and concluded that the high moisture control reduced the sclerotial growth. Robin and Phookan (1993) have
observed the survival of *S. oryzae* under humid condition of soil of Northeast India. Hannusch and Bokand (1966) have reported the influences of air temperature and relative humidity on biological control of white mole of bean caused by (*Sclerotinia sclerotiorum*). Shukla (1972 a) reported that wilting of Yuar plants caused by *O. texana* Var. *parasiticum* was more in sandy soil and least in clay soil. The percentage of infection decreased as the quantity of sand decreased in the soil vice versa. Yasmin et al. (1988), have studied the collar rot of maize caused by *S. rolfsii* in relation to soil composition. Sugha et al. (1993) has investigated the factor affecting development of collar rot of gram caused by *S. rolfsii* (Devi and Singh 1999).

**(d) Application of soil pH**

On the basis of observational data it has been seen that the interval of pH values such influence the percentage infection of rot disease in paddy. A similar observation for root, rot (*P. comnivorum*) of cotton was made (Anonymous 1926) in texus and it was inferred that the disease could be checked in soil b changing there pH to a higher level of alkalinity. Grover and Chona (1960) also reported that the pH of the soil executed a strong influence on the infection of potato plants by *S. rolfsii*. They observed that between pH 4.8 to 7.3 the infection was about 100 percent and at pH 8.6 the percentage of infection was 40 to 50 percent only. But at pH 9.4 the infection was reduced to 10 percent Mathur and Sinha (1968) observed that root rot of Gour and wilt of Gram caused by *S. rolfsii* was maximum in acidic soil and minimum in alkaline soil.
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Similar observation were also, studied by Singh and Malhotra (1994) under the effect of temperatures and pH on growth and sclerotia of *R. solani* causing web blight of winged bean.

(5). **Quantitative analysis of other soil microorganism and there effects on sclerotial mortality.**

It was observed in the present investigation that increase in population of biotic antagonists like *Penicillium, Fusarium, Trichoderma*, mycorrhizal fungi, bacteria like *Pseudomonas* and Actinomyces in stem rot infested sick soil significantly reduced the sclerotial population of *S.oryzae*. This phenomenon can be considered as an application of two principal contradictory but not mutually exclusive explanations as suggested by Lockwood (1977) and Watson and Ford (1972). In a soil at steady state nutrient reservoir or inputs are consumed as they appear. The above saperophytic soil biota, thus compete intensely for nutrient and sclerotial propaguler can, not obtain the necessary energy for their germination. Although the sclerotial of *S.oryzae* are nutrient independent and can germinates in distilled water in the absence of exogenous nutrient as explained in previous experiments. According to the modified nutritional hypothesis of Ko and Lockwood (1967), under the action of soil biota, the endogenous nutrients of sclerotia are rapidly exuded leached and consumed. There is a sink in soil, which exhausts both external and internal nutrient reserves of the propagules and inhibits its germination. This may be one of the major reasons for the gradual decline of viable sclerotia of *S.oryzae*
during the course of investigation between 1998-2000. The other plausible reason for the inhibition of sclerotal propagules may be due to toxic chemical secreted by the above microbial population. Further, Hora and Baker (1971, 1974) have reported the existence of a volatile fungistatic factor in numerous soil particularly those, which are alkaline or neutral and other liming. Therefore the amount of inhibition or degeneration of sclerotal propagules of *S. oryzae* in the soil is the result of multiple inhibitory or stimulatory interactions, accordingly as the magnitude of the cumulative effects of unfavorable factors, there is a gradual decline of pathogen population. Thus the state of inhibition of *S. oryzae* propagules in rice soil is presumably governed by a balance between both groups of factors nutrition sink and toxic chemicals (Diem 1975). So it is suggested that if the sclerotia of rice soil are manipulated by introducing the hazardous organism like *Penicillium, Fusarium, Trichoderma* mycorrhizal fungi, *Pseudomonas* and actinomyces for inducing the fungistasis phenomenon and not allowing them to germinates during the most vulnerable stage of the rice crop. It serves as a passive method of control of the stem rot disease by reducing the sclerotal population and avoiding the infection. In fact the fast growing and antibiotic producing fungi *Trichoderma* spp are now known to control *Sclerotium rolfsii* in field condition (Wells et al. 1972). *Trichoderma viridis* and *penicillium frequentants* are able to control *Phytophthora* infection of best seedling (Liu and Vaughanc 1965). The use of actinomycetes as lytic antagonists has also been suggested by Mitchell and Alexander (1962), Vruggink (1970) and Arjunaro (1971) in the group of bacteria different strain of *Pseudomonas* were used with partial success by *Fusarium* and
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*Vaerticillium* (Luckwood 1968). The impact of mycorhizal fungi on different plant disease has been delineated in detail under review of literature. The evidence of the close correlation between antibiotic production and elimination of plant pathogen is discussed at length Mitchell (1973).

(6). **Effect of soil pollutants on test pathogen**

(i). **Effect of pollutants on colony growth of Sclerotium oryzae and some dominant, rhizosphere fungi.**

All the fungi showed inhibition after exposure to ammonia but *Sclerotium oryzae* showed stimulation after 10 min exposure on 72 hr. of incubation and *Penicillium decumbense* after 24 hr. up to 48 hr. of incubation after which the growth was inhibited. The inhibition of radial growth may be due to toxic effect of ammonia. Gilpatrick (1969 a,b) Pavilica et al. (1978) have also reported that toxic effect of ammonia to fungi. The increase in radial growth of the soil species might be due to stimulatory effect of ammonia. Low concentrations of ammonia are known to stimutal the germination of conidia (Schippers et al 1982 Laffler Schippers 1984). Nevertheless, was found by Setua and Sammader (1980) that the sensitivity of organism to ammonia was different from one another. Inhibition in radial growth to all fungi at higher dose might be due to fungistatic nature of ammonia. (Ko and Hora 1972, Setua and Sammader 1980, Schippers et al. 1982.

Inhibition of radial growth of fungi was seen in case of all the treatment with sludge, which may due to toxic substance present in sludge. Dowing 1971 observed effluents to inhibits the microbial population in
activated sludge treatment. Leach et al. (1978) also noted suppressed microbial activity. Varilin and Vasilliev (1983) have proposed a biological treatment model with ecosystem adoption at multi component sewage treatment specific microflora at each treatment.

The maximum grow stimulation was noticed at lower concentration of the cement dust pollutant. All the fungus, which are taken at present investigation showed inhibition at higher concentration of the cement dust and present inhibition decreased the claspo of time. The inhibition of fungi might be due to the neutralized of fungus character. But exact mechanism of the stimulation has not been clear at present investigation, which needs to do the research of further passage. In the present investigation it had been observed that lower concentration of the pollutant show that maximum radial growth comparison the higher concentration. Gradually when the concentration of the pollutant increase the growth of the fungus decrease due to toxicity of the pollutant. This parallel observation has been proved time by various researchers.

(ii). Effect of Pollutants on percent germination of sclerotia of Sclerotium oryzae and conidia of some dominant rhizosphere fungi

The germination of conidia of Aspergillus niger Nigrospora and Fusarium oxysporum was inhibited at 1000 μg/ml of sludge. The inhibition increased at higher concentration. Sludge however contain undesirable chemical, which may be toxic. The sclerotia of test pathogen also show similar pattern to that of other rhizosphere fungi, Downing (1971) also observed effluent to inhibit microbial population. The conidia of other
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rhizosphere fungi strictly checked due to increased of row sludge concentration. The inhibition of the germination of sclerotia and conidia due to toxicity of the component this evidences was finding by other researcher.

The maximum inhibition for 10 min exposure to ammonia was recorded for *Fusarium oxysporum* followed by *Trichoderma harzianum*, *A. niger*, *P. granulatum*, *Nigrospora*, *P. citrinum* etc. and for 30 min exposure the maximum inhibition was recorded for *Trichoderma harzianum*. Schipper *et.al* (1982) also reported that ammonia was responsible for the inhibition of conidial germination. The fungicidal action of ammonia on various gerera of fungi has been reported by numerous author (Zentmyer 1963, Henis and Chet. 1968; Tsoa and Oster 1981).

The sclerotial inhibition of the *Sclerotium oryzae* was also show the lower to higher exposure of time against the ammonia due to reason that toxicity of them.

Cement dust was found to inhibit the conidia of *Fusarium oxysporum* and *Sclerotium oryzae* at all the concentration whereas *Aspergillus fumigatus* showed least inhibition. The inhibition of conidia and sclerotia might be due to the toxic effect of cement dust. The responses of pollutant depend upon the microbial species to their resistant nature and concentration of pollutants, host species and environmental factor.

(6). **Germination of Sclerotia in the leaf exudates of twelve rice varieties**

The total sugar content was very low in the highly resistant variety such as Prasad, Saket and Ratna in comparison to other susceptible variety. The total sugar content was found to be highest in Basmati (22µg) and
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lowest in Prasad (2μg). These varieties are group into two in term of total sugar content in the leaf exudates sclerotial germination and pathogenicity. The spore germination and susceptibility were directly correlated to the total sugar content in the leaf exudates. Though the amino acid could not be analyzed quantitively the paper chromatography study ascertained that L glutamine present in Govind, Indrason, IR 36, Jaya, Manahar and L prolin present in Basmati, Indrasan IR 36, IR 50, Jaya, Manshuri, Panthdhan 4, have same.

Conductive role in the spore germination and indirectly help in disease development in 15 days old seedlings the presence of L Histidine in resistant varieties such as Prasad, Saket and Ratna and its absence in susceptible varieties indicate that L histidine is probably responsible for non germination of sclerotia in the highly resistant and resistant variety. The increased seedling infection at two weak stage as reported by Khan and Liby (1958). Kaur and kaur (1973) may be due to the presence of L glutamine and L proline and increased sugar as is evident from present investigation. Otani (1952) and Otani (1953) reported that sugar and some amino acid found to be best source for germination of spore. Otsuka et. al (1965) used 32 different carbon compound and 47 isolates and reported that the sugar and some amino acid were found to be influence the blast fungus. Mathur et.al. (1967), studied the effect of amino acid nutrition on the growth of races and good nutrient to other races. Riberio et.al (1976) observed low fungi toxicity for sclerotial germination of P. oryzae in rice leaf exudates. Saxena (1978) reported the presence of glutamic acid with other amino acid in the exudates of susceptible and resistant rice variety, CO 13 and CO 29 respectively. C.R.
Mohantly and S. Gangopadhyay also reported the presence of L Histidine in resistant variety Zenith, Tetep, Tadukhan and mention that the amino acid check the sclerotial growth. So present investigation also correlate the finding of Saxena (1978) and Gangopadhyay (1981) in cause of susceptible variety and bring forth the information that at seedling stage in rice is due to excessive sugar concentration and presence of L glutamine and proline in the leaf exudates of two weak old seedlings and presence of L Histidine in the leaf exudates of resistant varieties are non conductive to germination of *P. oryzae* spore. Same observation has been also reported by Mohanty and Gangoaphdhya (1981).

(7). **Application of Fungicides**

**In Vitro Condition**

In present investigation screening of twelve fungicides at 25, 50, and 100 against the fungus showed that Dithane Z 78, Dithan M 45 and capton at 25 and 50 ppm among the fungicides did not show adverse effect on the fungus. Consequently Diethan Z 78, Dithan M 45 at 25 and 50 ppm in different combination were tried in developing the selective medium. Dithan Z 78 and Dithan M 45 are brand spectrum fungicides and are effective against a number of plant pathogenic genera belonging to almost all the major group of fungi, (Holemes 1971). But these were not effective against the fungus at 25 and 50 ppm. Dithan Z 78 inhibited the mycelia growth of the fungus at minimum concentration of 600 ppm and not at lower concentration of the fungicides (Mishra and Das. 1967), which support the present investigation. However the ED 50 value of less than 5 ppm of this fungicides has also been reported against the fungus which is contrary to the
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present observation. Dithan M 45 showed Ed 50 value more than 100 ppm against the fungus when incorporated in the medium indicating that it also does not inhibits the fungus growth at lower concentration (Sharma and Mehrotra 1985) and supports the present finding.

In a combination study the minimum fungus growth occurred in the medium when 50 ppm of Dithan M 45+Dithan Z 78 was incorporated. The next treatment, which gave better growth of the fungus than the former treatment were Dithan M 45+Diethan Z 78 at 50 ppm. The next treatment which support the fungus growth more than the former treatment and at par with each other.

The growth on these, treatment did not differ significantly form each other. Diethan M 45 and 25 ppm gave significantly less growth than control but was least effective on inhibiting, the growth in comparison to other treatments. These combinations has been used by several workers as a supplement in the medium to restrict the colony diameter of the test fungus and other genera of fungi from spreading (Martin 1950, Synder et.al 1959 and Singh and Mitchell 1961) which also support present observation.

In Vivo Condition

In the tabulated data it has been seen that five fungicides, which are found mostly effective in laboratory, list were much effective against the fungus growth. (Table ) Bavistine and Topsin-M gave the minimum disease index and maximum increase in yield over control. Mishra and Das (1967) also evaluate the fungicides for the control of stem rot of paddy and reported similar result, which are parallel to the present investigation. Diethan Z-78 gave significantly higher disease and lesser yield than the Bavistine and
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Topsin-M. Brassicol was found to be best effectiveness in which disease index and yield were less comparison to other four fungicides, which are taken in the present investigation. Emission was found to be next effectiveness than the Topsin m and Bavistine was more effectiveness than the Brassicaol, which was proved to be least effectiveness against all the five fungicides. Singh and Pavigi (1969) also reported that the stem rot of rice caused by Leptosphaeria salvinii against these fungicides and was found to similar result that are parallel to the my investigation. Among the fungicides Bavistine and Topsin M have earlier been reported effective against the fungus (Jain 1971).

Efficacy of various fungicides in vivo condition indicated that Brassicol though an effective fungicides in vitro performed poorly in vivo. Jain (1977) also observed that Brassical was not of much help in controlling stem rot of rice effectiveness of Bavistin and Topsin M may be due to their systemicity which are translocated through the system at the site of infection of the fungus and may have the curative effect on rice plant.