STUDIES ON THE EFFECT OF ORGANIC AND INORGANIC AMENDMENTS ON THE SOIL AND RHIZOSPHERE MICROFLORA IN RELATION TO THE BIOLOGY AND CONTROL OF SOIL-BORNE PLANT PATHOGEN (*Sclerotium rolfsii Sacc.*)

ABSTRACT

PRONOY RANJAN DEB

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF **DOCTOR OF PHILOSOPHY IN BOTANY**

To

DEPARTMENT OF BOTANY
SCHOOL OF LIFE SCIENCES
NORTH-EASTERN HILL UNIVERSITY
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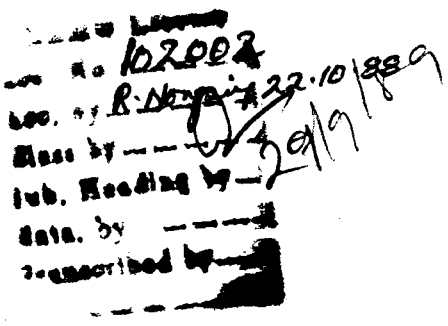


ABSTRACT

In the modern system of soil-borne disease management, control with the help of soil amendments is comparatively a new approach. The soil amendments may reduce the disease severity either by inhibiting the growth of the pathogen or by stimulating the antagonistic soil and rhizosphere microflora. The fungal propagules are known to remain dormant in soil, which may germinate due to break of "imposed dormancy" (i.e. due to soil fungistasis) following soil amendments and may increase or decrease the disease severity. Therefore, it is important to develop methods, to reduce the number of propagules of the pathogen in soil below a dynamic damage threshold level. In the present investigation "Studies on the effect of organic and inorganic amendments on the soil and rhizosphere microflora in relation to the biology and control of soil-borne plant pathogen (**Sclerotium rolfsii** Sacc)", emphasis has been given to control the pathogen (i.e. **S. rolfsii**) with the help of some easily available green plant materials, and with some organic/inorganic chemicals (i.e. inorganic fertilisers, antibiotics and fungicides).

The thesis is presented under following headings:

General Introduction, Review of Literature, Environmental features with General materials and methods, Experimentals (eight chapters), General Discussion, Summary and References. Each chapter has an Introduction, Materials and Methods, Results and Discussion. The experimental work has been divided

into two parts. Part one "Soil Survival" with one chapter i.e. Studies on soil fungistasis, while Part two "Plant soil relationship" consists of seven chapters viz., (1) Effect of organic, (2) inorganic amendments on the soil, rhizosphere microflora, disease development and yield, (3) effect of nitrofurans, (4) antibiotics, (5) fungicides on the rhizosphere microflora, disease development and yield, (6) studies on antagonism and biological control of **S. rolfsii** and (7) studies on the effect of organic and inorganic amendments to soil on rhizosphere microflora and disease development in maize.

Part - I: Soil fungistatic activity against **S. rolfsii** was studied in relation to seasonal variation, microbial population, available nutrients and microbial activity (i.e. dehydrogenase) in three different soil types (i.e. forest, garden and grassland soil). It has been observed that soil fungistasis is seasonally variable i.e. with an increase in summer months compared to the winter. No correlation was observed between the seasonal variation of the factor and of microbial population in the soil in general, but a direct correlation with available nutrients (i.e. total sugar) in soil has been observed. Among the physico-chemical factors, soil moisture and enzyme activity shows a direct relationship with the seasonal variation of soil fungistasis. Ethrel (2 chloro ethane phosphonic acid), an ethylene generator in aqueous solution, was found to induce fungistasis in sterilised soil which was otherwise non fungi-

static. It inhibits the sclerotial/spore germination in soil, in aqueous solution and affect soil microbes even at lower concentration (1 µl/L). The inhibition due to ethrel was reduced by supplementing glucose (1% and 10%) in experimental soil. This supports the inhibitor and stimulator theory to balance soil fungistasis proposed by Smith (1973). The probable pathway and the factors involved in soil fungistasis has been discussed.

Part - II: Among the organic amendments, **Eupatorium adenophorum** followed by **E. riparium** leaf extract, inhibited the growth of **S. rolfsii in vitro**. The inhibition in sclerotial germination is positively correlated with the concentration of the plant extracts used. Viability of the sclerotium in soil decreased (excepting **E. adenophorum**) with the increase in concentration of the amendments applied and with time. Organic amendments did not have any adverse affect on soybean seed germination. Amendments significantly increases total phenolic compounds in the treated soybean radicles **in vitro**.

All the soil amendments stimulated fungi, actinomycete and bacteria (excepting **E. adenophorum** and **E. riparium**) in soil, whereas, a significant increase in bacterial population was observed in soybean rhizosphere. Aspergilli, mucorales, **Trichoderma** spp. were found to be stimulated in soil and rhizosphere. A comparatively higher population of Aspergilli was observed after the application of **Helianthus annuus** and **Pinus**

kesiya amendment to soil and **E. adenophorum** (3% w/w), amendment in rhizosphere. **Trichoderma harzianum** and **T. koningii** were found to be stimulated in the soil and soybean rhizosphere following **E. riparium** soil amendment. A total of thirty eight and thirty two species belonging to eighteen and fourteen genera have been isolated from soil and rhizosphere, respectively.

Soil amendments (i.e. **P. kesiya, H. annuus,** Poultry litter) initially increased **S. rolfsii** population in soil, which subsequently declined. While **E. adenophorum** amendment to soil reduced the population. Although, pre-emergence seed rot was observed, but reduction in disease severity was achieved with all the soil amendments. Maximum reduction in disease severity was observed with **E. riparium** amendment.

Organic amendments to soil in general did not produce any adverse affect on the growth of soybean plants. Infact, **H. annuus** and Poultry litter amendment increased the growth significantly. All the amendments increased the dry weight of shoot, pod and yield in soybean plants. Comparatively higher yield was recorded with **E. riparium** (3%w/w) and Poultry litter (2%w/w) amendments. Under field condition higher concentration (40q/ha) of the soil amendments reduced foot rot disease together with increased soybean yield.

Among the inorganic amendments, urea and zinc sulphate (0.25, 0.5, 1.0%w/w) suppressed the growth and sclerotial

germination of the pathogen **in vitro**. The survibality of sclerotium in soil decreased with time and concentration of the chemicals. Higher concentration of inorganic amendments decreased soybean seed germination **in vitro**. Significant increase in phenolic compounds was observed in the treated soybean radicles. Soil fungal population increased due to rock phosphate and ammonium nitrate amendment to soil. But rock phosphate (0.1, 0.25%w/w) only increased the fungal population in soybean rhizosphere. A significant increase in bacterial population in soil and rhizosphere was recorded with all the inorganic amendments used. Although increased actinomycete population was observed in soil but the population decreased in the rhizosphere. Soil amendments stimulated mucorales in soil and mucorales and Aspergilli in the rhizosphere. Zinc sulphate (0.25%w/w) boosted Aspergilli in soil whereas, rock phosphate (0.25%w/w) and urea (0.5%w/w) increased the same in the rhizosphere. Calcium nitrate (0.25%w/w) and zinc sulphate (0.1%w/w) stimulated penicillia in soil and rhizosphere of soybean seedlings respectively. A total of twenty seven and thirty one species belonging to seventeen and fifteen genera have been identified and isolated from the amended soil and rhizosphere respectively.

Amendments initially increased the pathogen population resulting an increase in pre-emergence seed rot. Significant increase in **S. rolfsii** population after urea amendment is probably, due to the breaking of "imposed exogenous dormancy"

by soil fungistatic factor present in the soil. The seedlings which escaped rot, delayed symptom expression by two weeks (0.5%w/w) concentration in all the cases) compared to infected control. Reduction in disease severity (i.e. 30-50%) was observed with all the inorganic fertilisers used.

Higher concentration (0.5% w/w) of most of the inorganic fertilisers were found to have toxic effect on the seedling growth (i.e. height) and yield excepting calcium nitrate and urea (0.1%w/w). Urea (0.1%w/w), calcium nitrate (0.25%w/w) and calcium carbonate (0.1, 0.25%w/w) increased the shoot weight, whereas, urea (all the concentrations) increased root weight. No significant increase in soybean yield due to the inorganic soil amendments was observed.

Under field condition a direct correlation was observed between the **S. rolfsii** population in soil and soybean foot rot in case of urea (80 kg/ha) at the initial stage. Although, zinc sulphate (5, 10kg/ha) initially increased the population but it declined slightly with time. Whereas, rock phosphate (40kg/ha) showed a gradual increase in pathogen's population. A significant reduction in disease severity was recorded with all the inorganic chemicals used, of which zinc sulphate gave the best result. Urea (80kg/ha) and Zinc sulphate (10kg/ha) stimulated the plant growth which was reflected on the increase in plant height, vigour and yield as compared to infected control and the lower concentration of the chemicals.

Among the three nitrofurans tested, furazolidone (at all concentrations) and nitrofurantoin (higher concentration only), completely inhibited the growth of **S. rolfsii in vitro**. Decreased sclerotial germination was observed with the increase in concentration of the chemicals. Complete loss of viability after 30 days of incubation was observed due to furazolidone (1000 µg/L) amendment to soil. Others also reduced the number of viable sclerotia with the increase in incubation period. Significant reduction in fungal and bacterial population in the soybean rhizosphere following the foliar application of nitrofurans was observed. All the nitrofurans reduced the disease severity (more than 50%). Better yield production was achieved with furazolidone treatment only.

Antibiotic viz. actidione and thiolutin completely inhibited the growth of **S. rolfsii in vitro** even at lower concentration (20 µg/L). Others showed slight inhibition. Loss in viability of sclerotium by 50% after 15 days and complete loss after 60 days of incubation in treated soil was observed with higher concentration of all the antibiotics tested.

The concentrations of antibiotics used here, however, found to be toxic to soybean seed germination, excepting few cases i.e. thiolutin (upto 80 µg/L), streptomycin (20, 40 µg/L) and penicillin (20 µg/L), whereas, streptomycin (all the concentrations), penicillin and chloramphenicol (20, 40 µg/L) stimulated the radicle growth.

A significant decrease of fungi, bacteria and increase in actinomycete population was recorded in the rhizosphere of soybean seedlings following foliar spray with antibiotics. A total of twenty nine species belonging to thirteen genera were isolated from the rhizosphere.

Higher concentration of all the antibiotics delayed the symptom expression. Actidione followed by penicillin and thiolutin gave better control of disease (at 100 µg/L) compared to others. Slight increase in yield was recorded with thiolutin, chloramphenicol (lower concentration), followed by streptomycin (all the concentrations).

Among the seven fungicides tested, PCNB and agrosan produced highest inhibition in growth of **S. rolfsii**. Viability of sclerotium reduced when dipped in aqueous solution of sulfex and agrosan, but in soil, dicloran and agallol was found to be the most effective. Sulfex, was found to be toxic to soybean seed and seeddlings while others produced slight inhibition in seed germination only. Soil drench with fungicides, decreased the microbial population (i.e. fungi, actinomycete and bacteria) in the rhizosphere. A total of twenty nine fungal species belonging to thirteen genera have been isolated, of which Aspergilli and mucorales were found to be the dominant.

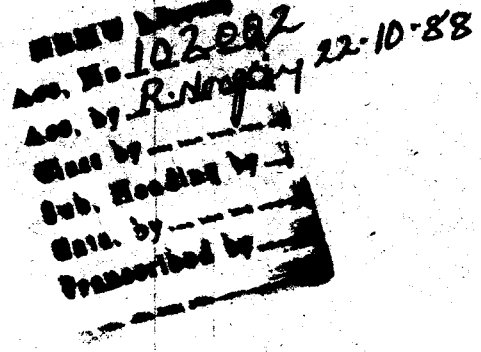
A delayed disease development was achieved in soybean plants grown in the infested soil drenched with PCNB and agallol. All the fungicides used have controlled the disease severity,

however., the best result was obtained with PCNB and agallol. Increase in yield was observed with PCNB and agrosan, irrespective of the concentrations used, while others also increased the yield but only at lower concentration. On the other hand delan and sulfex reduced the yield considerably.

In biological control studies, eleven dominant rhizosphere fungi of soybean were selected to determine their activity against the growth of **S. rolfsii in vitro** and **in vivo. Trichoderma viride** and **T. koningii** showed volatile, while **T. viride, T. koningii, T. harzianum** and **Aspergillus flavus** showed non volatile antibiotic activity resulting inhibition in growth of **S. rolfsii**. The inhibition rate decreased with the increase in incubation period. These three **Trichoderma** spp. also parasitized **S. rolfsii** mycelium through coiling, lysis, penetration, growth, conidia formation and ultimately bursting the host hyphae. A necrotrophic mycoparasitic activity of **S. rolfsii** on **Aspergillus niger** was also observed. Others show mutual intermingling growth **in vitro. T. viride** and **F. solani,** also produced inhibition zone (type C) in some cases.

Trichoderma harzianum and **T. koningii** amendment to **S. rolfsii** infested soil gave the best control of soybean foot rot disease. Population of **S. rolfsii** decreased considerably when the antagonist cultures were (i.e. **Trichoderma** spp.) separately amended to soil, which could be correlated with the decrease in disease severity. Higher yield of soybean was recorded with **Trichoderma** spp. compared to others.

Sclerotium rolfsii isolated from infected maize (**Zea mays** L) cobs differ morphologically from the soybean isolate. The isolate grew very fast producing large number of small brown sclerotia. **E. adenophorum** and **E. riparium** soil amendments reduced the **S. rolfsii** population, while urea had virtually no effect. All the soil amendments used reduced the disease severity in maize plant.



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I certify that the thesis entitled "STUDIES ON THE EFFECT OF ORGANIC AND INORGANIC AMENDMENTS ON THE SOIL AND RHIZOSPHERE MICROFLORA IN RELATION TO THE BIOLOGY AND CONTROL OF SOIL-BORNE PLANT PATHOGEN (i.e. ***Sclerotium rolfsii*** *sacc.)" submitted by Mr. P.R. Deb for the Degree of* ***Doctor of Philosophy*** *of the North-Eastern Hill University embodies the record of the original investigation carried out by him under my supervision. He has been duly registered, and the thesis presented is worthy of being considered for the award of the Ph.D. Degree. This work has not been submitted for any degree of any other University.*

Place: **Shillong**
Date: 21st January '87

(B. K. Dutta)
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DEDICATED

TO

MY PARENTS

CONTENTS

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INTRODUCTION AND SCOPE OF WORK

Sclerotium rolfsii Sacc. is a dominant soil-borne pathogen of several economic crops of the tropical and sub-tropical regions of the world. This fungus has been reported to attack 189 species of plants distributed among 42 dicotyledons, monocotyledons and ferns (West, 1961). The fungus occurs widely in tropical soils and is a major contributing factor for poor yields in several agricultural areas.

This fungus is also dominant in the North-Eastern Region of India (Meghalaya), causing diseases in many agricultural crops viz. soybean, maize, etc. (Maiti, 1978) and naturally deserves more attention. Meghalaya represents areas ranging from tropical plains to temperate hills with high rainfall and humidity having unique climatic conditions for the growth of the vegetation and which also favours infection of the plants by a variety of plant pathogens (i.e. **S. rolfsii**) causing serious diseases.

Much information concerning organic and inorganic amendments to soil with special reference to disease control is available (Leach and Davey, 1942; Chowdhury, 1946b; Papavizes **et al**, 1968, 1970; Srivastava and Sinha, 1971) but the mechanism of reduction of disease severity is not properly known. The effect of organic and inorganic amendments to pathogen may be either direct due to their toxic effect (Leach and Davey, 1935; Henis and Chet, 1968; Chaudhuri and Maiti, 1978) or indirect by influencing antagonistic microflora (Linderman

and Gilbert, 1973a,b; Dutta and Isaac, 1979a,b) or may be due to their effect on the interaction between the pathogen and microorganisms in soil and the rhizosphere. Therefore, an attempt was made to study the possible effect of organic and inorganic soil amendments, to the soil and rhizosphere microflora in relation to disease development in some crop plants (i.e. soybean and maize) grown in **Sclerotium rolfsii** infested natural soil.

Many promising fungicides active against **S. rolfsii in vitro**, have been evaluated for their performance in green house and in the field. The method of application includes seed treatment, soil drench and rarely by foliar spray. Only PCNB was found to be most effective among the fungicides, which inhibits the pathogen, resulting in considerable reduction in the disease severity. Benodanil was also found to have cidal affect (Pan and Sen, 1977), but due to its high cost its application is limited to glass house crops only. In the present study, importance was given to disease control in soybean with special reference to rhizosphere microflora, following soil drench with some fungicides and foliar spray with other chemicals.

The ability of soil-borne plant pathogens to infect plant, depends on their survival and germination of the spore/resting structures. In the study of survival of soil-borne plant pathogens, antibiosis and fungistasis are two

important phenomena, which should also be taken into consideration for the control of soil-borne plant pahogens (Dutta, 1981). Therefore, an attempt was made to determine the interaction of **S. rolfsii** with some fungi isolated from the experimental soil and soybean rhizosphere. Experiments on the effect of fungistasis with reference to the survival of the pathogen (i.e. **S. rolfsii**) was studied in relation to the soil factors i.e. physico-chemical properties, total microbial population, total sugars and other possible factors (i.e. ethylene) involved in it. Although **S. rolfsii** is the main fungus under study, the author has used **Fusarium solani** as a contrast to evaluate the role of ethylene in soil fungistasis.

The problem will be approached mainly under the following headings:

i) Effect of organic (**Eupatorium adenophorum, E. riparium, Pinus kesiya, Helianthus annuus** and Poultry litter) and inorganic substances/chemicals (i.e. urea, rockphosphate, zinc sulphate, calcium carbonate, calcium nitrate, ammonium nitrate, antibiotics, nitrofurans and fungicides, etc.) on the growth, survival and development of **S. rolfsii in vitro.**

ii) Effect of organic and inorganic amendments on the soil and rhizosphere microflora of the host plants (qualitative and quantitative changes).

iii) Effect of chemicals (i.e. antibiotics, nitrofurans

and fungicides) application (foliar/soil drench) on the rhizosphere microflora of soybean (qualitative and quantative changes).

iv) Effect of organic and inorganic amendments to soil on the growth, disease development and yield of the host plants (i.e. soybean and maize) grown in the **S. rolfsii** infested natural soil.

v) Studies on the seasonal variation of soil fungistasis in relation to physico-chemical factors, microbial population and nutrient in different soil types.

vi) Studies on the microbial antagonism against the growth and survival of **S. rolfsii** and their consequent prospect for the control of foot rot disease in soybean.

REVIEW OF LITERATURE

Sclerotium rolfsii Sacc. has caused losses to cultivated crops in the warmer regions of the Globe for centuries. The investigation of Rolfs in Florida before 1900, showed that the fungus, although unnamed at that time, caused serious blight of tomato and other plants. Saccardo (1911) named the fungus as **S. rolfsii**. Foot rot or Sclerotial wilt caused by **S. rolfsii** Sacc. (= **Corticium rolfsii** Sacc.) is known to occur in India as far back as 1915 when Shaw and Ajrekar (1915) reported this pathogen under the name of **Rhizoctonia distruens** from Bengal (now Bangladesh). Subsequently, it has been reported from Madras (Sundararaman, 1928), Bombay (Uppal, 1928, 1930), Madhya Pradesh (Dastur, 1935), Assam (Chowdhury, 1944a, 1945).

The host plants are susceptible at any stage of their growth. The fungus attacks the plant at collar region and below the soil. No positive correlation was observed between pH of the soil and incidence of disease (Chowdhury, 1946a). The optimum temperature required for the growth and sclerotia formation of **S. rolfsii** was found to be 28 - 30°C (Chowdhury, 1948b).

The fungus **S. rolfsii** Sacc. was first observed by Peter Henry Rolfs (1892) in the United States and there after a considerable amount of literature has been accumulated and extensively reviewed by Aycock (1966). Since, the present investigation relates to the effect of amendments on soil

microbes with reference to **S. rolfsii** and its survival, attempt has been made to cover the literature pertaining to the effect of organic and inorganic amendments on soil microbes in this review.

Organic soil amendments

Papavizas and Davey (1960) reported a substantial increase in the total number of soil and rhizosphere micro-organisms (fungi, actinomycetes and bacteria) and a suppression of the **Rhizoctonia** disease of bean, by the amendment of soil with green plant materials.

Mitchell and Alexander (1961) observed that the addition of small quantity of chitin to soil resulted in a marked reduction in the severity of root rot of bean caused by **Fusarium solani, F. phaseoli.** Chitin amendment was also found to be effective in reducing the vascular wilt of radish caused by **F. oxysporum.** On the other hand, various other carbon and nitrogen sources as well as chitin degradation products, gave little or no reduction in the root rot of bean.

Crop residues stimulate the activity of **Sclerotium rolfsii** in soil, possibly through its high competitive saprophytic ability (Cooper, 1961; David and Rao, 1965; Sengupta and Roy, 1971). Oat, alfalfa and corn residues instead of stimulating the growth was found to inhibit **S. rolfsii**

Mixon (1965, 1967) and Mixon and Curl (1967) observed reduction in growth rate and severe mycelial degradation, when oat residues were amended in sterilised and natural soil respectively, in comparison to growth rates with clover, peanut vetch or corn residues. This degradation or reduction is reported to be due to an increase in **Trichoderma viride** and bacterial population.

Henis **et al** (1967) reported that chitin similar to other organic amendments caused an increase in the soil microbial population, especially actinomycetes.

Manuring has also been reported to reduce disease intensity caused by **S. rolfsii.** Farm manure (compost) appeared to reduce rot of **Cyamopsis psoraloides** and **Cicer arietinum** significantly (Mathur and Sinha, 1970).

Sneh **et al** (1972) observed a correlation between the inhibition of the saprophytic activity of **R. solani** in chitin amended soil, and an increase in antifungal activity of n-butanol soil extract as compared to non amended soil.

Jordon **et al** (1972) reported that the germination of conidia, microsclerotia and the mycelial growth of **Verticillium dahliae** in soil, were inhibited by the addition of chitin, laminarin, wheat straw and oven dried green clover as soil amendments. They further observed a significant

decrease in the number of viable propagules of the pathogen counted from the soil amended with chitin and laminarin as well as a reduction in the disease severity of straw berry plants. They also observed an increase in bacteria and actinomycete in the rhizosphere of plants grown in chitin amended soil compared to the rhizosphere of those grown in natural soil.

Application of oil cakes of neem, groundnut, castor and ammonium sulphate increased the fungal population in the rhizosphere of egg plant. Oil cakes also adversely affected the frequency of parasitic fungi such as **Colletotrichum atramentarium**, **Rhizoctonia solani** and **Fusarium** sp. (Wajid Khan **et al**, 1974). Stem rot of sunflower caused by **S. rolfsii** was also reduced by oat straw, followed by castor and **Azadirachta indica** oil cake amendments (Gautam and Kolte, 1979).

It was also reported that the leaves of **Vinca rosea** accumulate more antifungal substances than flower, stem and root, which was found to inhibit **S. rolfsii** and other genera of fungi (Narain and Satapathy, 1977).

A complete control of **S. rolfsii** was achieved through different plant extracts viz. **Tinospora cardifolia** stem and root (Pariya and Chakravarti, 1977), **Datura**, **Cannabis**, **Thuja** and **Eucalyptus**, offered control of disease in pot as well as in field condition whereas tobacco though found

effective in pot experiment did not show complete control under field condition (Singh **et al**, 1979). Singh **et al** (1979) also observed that garlic leaf extract inhibited the growth of **Fusarium oxysporum** and **Sclerotium sclerotiorum** and produced disease free gram seedlings when incorporated in soil.

Maiti (1977) observed that incorporation of dried crop residues of rice, triticale, wheat and green betlevine stem in moist soil (50% WHC) lead to an increase in the activity and the population of sclerotium upto 3 folds. Beute and Rodriguez-Kabana (1979a) showed that wetting of dried green peanut stem, leaves and other crop produced primarily methanol, that stimulated the germination of sclerotia. They suggested that an enzymatic release of methanol by the action of hydrolases on pectin triggers germination of sclerotium (Beute and Rodriguez-Kabana, 1979b). Burying sclerotia with undecomposed plant tissue was reported either to decrease (Sonoda, 1978) or to increase (Beute and Rodriguez-Kabana, 1981) the total number of sclerotia. They also observed that the volatile active fraction was found to be an essential oil, exhibiting fungicidal activity which remains active against heavy doses of inoculum even at its minimum inhibitory concentration (0.05%) against **Helminthosporium oryzae.**

According to Fawcett and Spencer (1970) phenolics are essential for disease resistance. But the absence of

phenolics in the oil of **Caesulia axillaris** suggest that metabolites of secondary importance too have a role in the resistance of plant to fungal pests (Pandey **et al**, 1982).

Survival

Higgins (1922) found that the sclerotia of **S. rolfsii** transferred to fresh media from old cultures were viable upto two years, whereas others (Povah, 1927; Nisikado and Hirata, 1937; Nisikado **et al**, 1938) reported the viability of sclerotia upto 5 years. The sclerotium has also been reported to have survived in decaying vegetable matter saturated with water, for several months (Harris, 1937). Chowdhury (1945) found that 60% of the sclerotia remain viable after two months at a depth of 4 inches. But in water the sclerotia remained viable several months without any injury.

Deep ploughing was recommended as a control measure for **S. rolfsii** and this probably involves the germinability of sclerotia. It was observed that, the sclerotia' could germinate only in surface soil and were not able to germinate in deep soil due to the lack of oxygen supply (Abeygunawardena and Wood, 1957b).

Smith (1972a,b,c) showed that drying and wetting reduced the population of viable sclerotia and thus promoted the biological control as a result of nutrient leakage, which makes the entrance for the growth of several antagonistic

microorganisms, probably the lytic soil organisms on the surface of sclerotia which finally lysed them. High moisture level in soil (80% WHC) also found to disintegrate the sclerotia and all of them disappeared in 40 days (Rana and Sengupta, 1975). They also proved the effectiveness of deep ploughing indirectly in the laboratory. It was however reported that the sclerotia produced in culture and the sclerotia produced in nature differ physiologically (Linderman and Gilbert, 1973b). The sclerotia of **S. rolfsii** produced in natural condition do not germinate on wetting as do the culture produced one (Beute and Rodriguez-Kabana, 1979b). A confirming evidence was given by them that the low disease intensity occurs in peanut, when rainfall follows a dry spell.

Survival of sclerotia in soil was also reduced by amending soil with alfalfa hay (Menzies and Gilbert, 1967). Similar results were also obtained when soil was exposed to the vapour of alfalfa distillate. Linderman and Gilbert (1973a,b) also observed that alfalfa distillate vapour stimulated the mycelial growth from the sclerotia, under natural soil. Vigour of mycelial growth from sclerotia markedly decreased in soil when the soil was exposed previously, to vapour from natural or synthetic mixture of alfalfa distillate. It was also proved that the decomposition of alfalfa residues produce ammonia which was found to be toxic to **S. rolfsii** (Sonoda, 1977).

An eruptive germination of sclerotia of **S. rolfsii** was also recorded when they were exposed to volatile compounds (alcohols and aldehydes) evolved from plant tissue (Linderman and Gilbert, 1969, 1973a,b; Beute and Rodriguez-Kabana, 1979a,b; Punja and Grogan, 1981a), following drying (Punja and Grogan, 1981a). Punja **et al** (1984) demonstrated that isopropyl and butyl alcohols were more stimulatory to germination than the volatiles evolved from dried and remoistened alfalfa leaf tissue.

Soil fungistasis

Survival of sclerotia in soil to some extent depend on the environmental factors. In natural soil, fungistatic factors generally inhibit the germination. The phenomenon of soil fungistasis was initially established by Dobbs and Hinson (1953). The term was applied to the inhibition in germination of fungal propagules in soil under apparently favourable condition but germinate when they were taken out and put in suitable substratum (Stover, 1958). This inhibition is due to the presence of inhibitory factor in the soil, which was described as fungitoxin (Hessayon, 1953), mycostasis (Dobbs and Hinson, 1953) and fungistasis (Jackson, 1957). According to Garret (1970) fungistasis is an 'exogenous dormancy' imposed upon fungal spores lying in soil, by factor/ factors that is/are wide spread in most of the soil, both natural and cultivated. Some workers believed that loss of nutrient from the spore results in inhibition of germination

(Ko and Lockwood, 1967). While to others, it is due to antibiotics production by soil microorganisms (Hora and Baker, 1972).

Fungistasis is a seasonally variable inhibitory factor of biological origin as evidenced by its absence in sterilised, deep subsoils and highly acidic soils (Dobbs and Hinson, 1953; Jackson, 1958; Dobbs and Bywater, 1959; Lockwood and Lingappa, 1963; Dutta and Isaac, 1979; Dutta **et al**, 1982). Some worker tried to correlate this phenomenon with soil pH (Schüepp and Frel, 1969), temperature and moisture content of the soil (Mishra and Pandey, 1974; Dutta and Isaac, 1979). This inhibition could be annuled with the help of exogenous supply of nutrient and by autoclaving the soil, suggests the death of the microorganism responsible for the production of the inhibitory substances. Griffiths and Dobbs (1963) also showed that the autoclaved soil released a high concentration of reducing sugar into the soil possibly as a result of partial hydrolysis of polysaccharide substrates, normally present in the soil and also as a result of the death of soil microorganisms.

Lockwood and Lingappa (1963) also showed that fungistatic activity could be reintroduced in autoclaved soil when inoculated with actinomycetes. They reported a wide variety of soil microflora responsible for fungistasis and is apparently more prevalent among actinomycetes than bacteria and fungi.

Smith (1973) found that ethylene is the main inhibitory substance that evolved from soil due to microbial activity. Top soils produced greater amount which was found to be more fungistatic compared to subsoils that produce lesser amount of ethylene (Dutta **et al**, 1982). Certain soil fungi (Ilag and Curtis, 1968) and soil bacteria (Chen **et al**, 1976; Primrose and Dilworth, 1976) produce ethylene in pure culture. It has been suggested that spore forming anaerobic bacteria (Smith and Cook, 1974) and fungus **Mucor hiemalis** (Lynch, 1975) constitute the most important groups of ethylene forming microbes in soil.

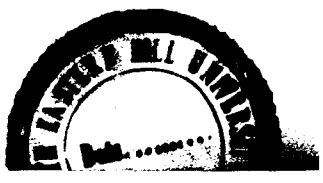
The modern concept of soil fungistasis is based on three general hypothesis.

The first hypothesis, preformed non volatile inhibitor, the evidence for this hypothesis has been given by Baisith and Vaartaja (1974) and Vaartaja (1967). The second hypothesis: preformed volatile inhibitor, (Balis and Kouyeas, 1968) show that inhibition due to allyl alcohol and other inhibitors involved in soil fungistasis are inorganic compound (Ko and Hora, 1971); an unidentified substance released by elevating soil pH (Hora and Baker, 1972), and ethylene (Smith, 1973). Third hypothesis involves inhibition resulting from microbial utilization of propagule exudates (Bristow and Lockwood, 1975).

Selective treatments of soil offer an approach to

the identification of the kind of organism responsible for ethylene production. Diffusion of oxygen (Smith and Restall, 1971; Smith and Cook, 1974) and the addition of ferrous ion to lower the oxidation-reduction potential of soil (Smith, 1976) stimulated ethylene production. On the other hand, nitrate (Smith and Restall, 1971; Smith and Cook, 1974) and manganese ions (Smith, 1976) which raised the oxidation-reduction potential, inhibit ethylene production. Many soil autoclaved at 121°c. no longer produced ethylene, but treatment of soil with moist heat at 80°c for 30 mins., failed to stop ethylene production when incubated at 25°c (Smith and Cook, 1974) and 30°c (Sutherland and Cook, 1980). Ethylene in soil, most likely produced by facultative or strictly anaerobic bacteria which were spore former or possibly the bacteria that isolated from heat treated soil (Sutherland and Cook, 1980).

Lewis and Papavizas (1975) stressed that energy source and other organic materials added to soil may reduce or completely annul fungistasis initially. After a week or more, a long term effect may become evident as manifested by an enhancement of fungistasis. Such increased fungistatic potential was observed after adding alfalfa hay to soil (Adams **et al**, 1968; Adams and Papavizas, 1969; Sneh **et al**, 1976) alfalfa distillate (Linderman and Gilbert, 1975), corn stover (Papavizas and Adams, 1969) and oat straw (Adams **et al**, 1968). According to Smith (1976) whether the surviving sclerotia of **S. rolfsii**



will germinate or not, depends upon the balance of nutrient stimulant and ethylene inhibitory action.

In a model fungistatic system, inhibition in germination was correlated with the increased loss of C^{14} labelled compounds from spore (Sneh and Lockwood 1976; Filonow and Lockwood, 1979, 1983) of which more than 90% of the carbohydrate was glucose.

The metabolic activity of soil microbes was mainly determined by the estimation of dehydrogenase activity in soil. Several workers used 2, 3, 5 TTC reduction method to equate the dehydrogenase activity with the biological activities of the soil (Lenhard, 1956; Stevenson, 1959). Stevenson (1959) found a correlation between the oxygen uptake and dehydrogenase activity in soil. He further observed that a close relationship exists between dehydrogenase activity and bacterial number in the decomposition studies. It was observed that the enzyme activity of the same soil differs with respect to different times (Ross and Roberts, 1970). Ross (1971) further suggested that dehydrogenase enzymes, possibly play an important role in the initial stages of the oxidation of soil organic matter of transferring hydrogen or electron from substrate to aceptor.

Skujins (1973) observed that the dehydrogenase activity could not be correlated with the microbial numbers. He further stated that an assay of dehydrogenase activity may be used

to predict the proteolytic nitrifying and respiratory activities in soil.

Inorganic Soil amendment & Chemical control

Waksman (1922) observed that the plots receiving mineral fertilizers applied separately or in various combinations with organic fertilizers gave higher population of fungi than did similar plots receiving no mineral fertilizers. The effect of mineral fertilizers (NPK) and soil reaction (pH) on the total number and types of soil fungi was determined in the laboratory as well as in the field by Kaufman and Williams (1964). They observed that nitrogen fertilization had the greatest effect on the composition of the soil fungus population of fallow laboratory soil, followed by phosphorus and potassium respectively. They reported from their field observation that only three microorganisms viz., **Myrothecium** spp., **Verticillium** spp. and **Penicillium purpurogenum** were affected by the application of nitrogen fertilizer.

Reduction in sclerotial germination with a wide array of nitrogenous compounds including ammonia, urea,ammonium nitrate, chitin, peptone, calcium nitrate and ammonium acetate at 0.2%w/w, has been observed by Henis and Chet (1968), and is due to the build up of antibiotic producing organisms in the mycosphere of sclerotia. Sclerotium germination of **S. rolfsii** in soil was inhibited by the lower level of nitrogen (50 ppm) in the form of ammonium or nitrate nitrogen. While higher

level (2000 ppm) was found directly toxic to the sclerotia. The effect of nitrate was not due to the changes in soil reaction or nitrite accumulation (Avizohar-Hershenzen and Shacked, 1969). **In vitro** test showed an irreversible loss in germination above 2000 ppm of ammonium and nitrite but effects with nitrate was reversible.

In pot trials 20 lb N/acre in the form of ammonium sulphate or urea, half before planting and remaining 15 days after seedling emergence, controlled wilt or collar rot of potato caused by **S. rolfsii** (Sulaiman and Rhode, 1968). Lower levels of calcium nitrate has shown partial effect. Field application of 160 Kg N/ha as calcium ammonium nitrate or ammonium sulphate or 200 Kg N/ha as urea significantly reduced root rot of sugar beet caused by **S. rolfsii** with increased yield (Thakur and Mukhopadhyay, 1972). Further increase in doses reduced the sugar content of the beet tubers. It was reported that nitrogen application (100 Kg N/ha) increases the susceptibility of egg plant to **Verticillium** wilt disease (Sivaprakasam and Rajagopalan, 1974).

The effect of six different combinations of inorganic fertilizers (calcium, ammonium nitrate, super phosphate and muriate of potash) and farm yard manure incorporated into soil, on the bacterial flora in the rhizosphere of paddy at the tillering, pre-flowering and the grain formation stages were observed by Mishra and Das (1975). They reported that

the bacterial population of the rhizosphere in both treated and untreated soil was significantly higher than that found in corresponding non-rhizosphere soils.

Ammonium and nitrate form of nitrogen, which inhibited sclerotial germination, however were not inhibitory to mycelial growth of the pathogen suggests a direct toxic action of nitrogen on sclerotial germination at high levels (Chaudhuri and Maiti, 1978). While other workers suggest that inhibition in sclerotial germination is due to either indirect by altering host susceptibility or population of antagonistic soil micro-organisms (Henis and Chet, 1968) or direct through the release of ammonia (Henis and Chet, 1967; Avizohar-Hershenzon and Shacked, 1969; Punja and Grogan, 1982; Punja **et al**, 1985).

Increased calcium levels in the tissue following application of calcium nitrate or sulphate may provide some level of control under low level disease condition (Punja **et al**, 1985). High calcium content in tissue may partially offset the action of oxalic acid and cell wall degrading enzyme (Punja **et al**, 1985). Application of hydrated lime did not give satisfactory control of disease in golf green (Punja **et al**, 1982).

Varieties of chemicals i.e. fumigants, fungicides and herbicides have been screened **in vitro** and **in vivo** against **S. rolfsii**. The fumigant mostly used as nematicides was

found to control **S. rolfsii** successfully. Result obtained till 1965, showed that chloropicrin (Mc. Clellan **et al**, 1947) methyl bromide (Munnecke and Lindgren, 1954) and chloropropene (Abeygunawardena and Wood, 1957a) are significantly effective and gave disease control. Results with vapum in disease control has been inconsistant (Aycock, 1966). Mc. Carter **et al** (1976), however obtained a significant reduction in the incidence of southern blight of tomato through its application. Chloropicrin and methyl bromide under plastic trap were moderately effective (Mc. Carter **et al**, 1976). Methyl bromide was effective against mycelial bits but less against sclerotia of **S. rolfsii** in a free flowing system (Munnecke **et al**, 1978). Sclerotia buried at 10 cm deep in soil were killed by methyl bromide at 500 Kg/ha but higher doses were required for greater depth (Hass, 1976). Rodriguez-Kabana **et al** (1976) observed that the nematocide ethoprop reduced the southern blight of peanut effectively by increasing **Trichoderma viride** and **Aspergillus** spp. population in the soil.

Herbicide dinoseb was found to be toxic to **S. rolfsii**, (Garren, 1959a) which was also used for elimination of weed hosts (Boyle, 1961). Paraquat (Rodriguez-Kabana **et al**, 1967a), atrazine compounds (Curl **et al**, 1968; Pitts, 1969; Bozarth and Tweedy, 1971 and Rodriguez-Kabana **et al**, 1967b, 1968), trifluralin (Rodriguez-Kabana **et al**, 1969) and EPTC (Peeples, 1970) affect the germination and growth of **S. rolfsii**

in different degrees. Sclerotia produced in the above mentioned chemical treated cultures were larger in size but their rate of production was reduced (Curl **et al**, 1968; Bozarth and Tweedy, 1971). Some herbicides have been shown to act by stimulating antagonists. Atrazine has been found to be stimulatory to **Trichoderma viride** in soil (Rodriguez-Kabana **et al**, 1967b). Similar results have also been obtained with EPTC (Peeples **et al**, 1976).

Sumithion among the nine insecticides tested **in vitro**, showed maximum inhibition in the growth, delayed formation of sclerotial initials and reduced the number of sclerotia (Reddy and Anil Kumar, 1975).

A large number of fungicides have been screened for their **in vitro** effect on the growth of mycelium and germination of sclerotia of **S. rolfsii** in culture. Many of the fungicides effective **in vitro** were found to be less effective or completely ineffective in the field. Pentachloronitrobenzene (PCNB) has been widely used for the control of diseases in ornamental and other important crops (Gould and Mclean, 1954; Gould, 1954a,b) at 200 lb/acre. The efficacy of PCNB to control the disease caused by **S. rolfsii** has been investigated extensively. Cooper (1956a) found that, PCNB was highly effective at 6-12 lb/acre.

Mishra and Chand (1970) demonstrated the effectiveness of PCNB in controlling the root and foot rot disease of wheat

caused by **S. rolfsii**. Similar results were also obtained by Mukhopadhyay and Thakur (1971) and Agnihotri **et al** (1975) in controlling root rot of sugar beet infected by the same pathogen. Chaudhuri and Maiti (1975) recommended higher concentration together with the successive doses of PCNB required for the successful control of this pathogen.

Other fungicides like Rhizoctol (Indulkar and Grewal, 1970; Bahadur **et al**, 1974; Khare **et al**, 1974), Panogen, Agrosan 5 W, ceresan W, as soil mixture gave considerable control to the diseases caused by **S. rolfsii** in several crops (Indulkar and Grewal, 1970). Captan, Thyram, copper carbonate EL 273, (Mishra and Chand, 1970; Khare **et al**, 1974) gave a considerable control in pre-emergence wilt of lentil and wheat. However, this result was not confirmed by Agnihotri **et al** (1975) against root rot of sugar beet. Ziride, Thiride I and II, Dithane M 45, Miltox (Chaturvedi and Agrawal, 1975; Bozarth and Tweedy, 1971) and Dichloran (Diomande and Beute, 1977) among the protectants were found to be effective against **S. rolfsii**. Higher concentration of vapam and formaldehyde (1000 ppm) were found highly effective in resisting growth of **S. rolfsii** and were recommended to protect the sugar beet against root rot caused by **S. rolfsii** (Mathur and Sarbhoy, 1980).

Systemic fungicides like benodanil (Maiti and Chaudhuri, 1975), vitavax and chloroneb (Mukhopadhyay and Thakur, 1971;

Agnihotri **et al**, 1975; Dasgupta, 1975) were found to be highly effective against **S. rolfsii.**

Application of benomyl controlled leaf spot of peanut caused by **Cercospora personata** and **Cercosporidium personatum** but increased stem rot considerably. Benomyl also imposed toxic effect on **Trichoderma** spp. Further, it prevented defoliation leading to the development of a good crop canopy which permitted humid condition in soil. This was found to be conducive for the stem rot development caused by **S. rolfsii** (Backman **et al**, 1975; Shew and Beute, 1984).

The method of applicaion included seed treatment, soil drench and rarely spray. Foliar spray of chemicals (fertilizers and fungicides) changed the rhizosphere microflora. It was reported earlier that foliar spray of urea increased bacterial population on wheat rhizosphere, whereas, fungal counts decreased (Vrany, 1963, 1965; Macura, 1971). Ammonium sulphate and 2-4D spray significantly increased the actinomycetes and fungal counts in the rhizosphere of **Eleusine coracana** (Bagyaraj and Rangaswami, 1982). The sprays also encouraged the establishment and multiplication of **Helminthosporium nodulosum** in the rhizosphere while aureofungin, benlate and disodium hydrogen phosphate sprays suppressed them.

Biological Control

The pathogenic root infecting fungi can survive saprophytically in soil in organic matter by competing with other

obligate saprophytes or on the dead host tissue, invaded during its parasitic phase (Garret, 1975). Barnett and Binder (1973) described two types of mycoparasitic activity i.e. necrotrophic and biotrophic based on the nutritional relationships. In former case, the development of the host is suppressed and the parasite can obtain its nutrients from the dead host, while in later case the parasite survives on the living host. Hyphal interference, penetration and coiling have been reported by several worker (Butler, 1957; Barnett and Lilly, 1962; Ikediugwu and Webster, 1970a,b; Dennis and Webster, 1971c and Skidmore and Dickinson, 1976) with different host and parasites. Several reports on the parasitic activity of **Trichoderma** spp and **Fusarium** spp. on other soil fungi are available but the nature of the parasitic activity of **Trichoderma** spp. on **S. rolfsii** has been worked out only in the recent years. **T. harzianum** isolated from soil, excreted β(1-3) glucanase and chitinase when grown on mycelium and cell walls of either **S. rolfsii** or **Rhizoctonia solani**. These enzymes degrade mycelial cell wall followed by penetration of the antagonistic fungus into mycelium of **S. rolfsii** as has been observed under scanning and transmission electron microscopes (Elad **et al**, 1983a,b).

The brown coloured sclerotia of **S. rolfsii** has melanized cell in the rind (Chet **et al**, 1967) which acts as an inhibitor of chitinase and glucanase, which are associated with the

biological degradation process (Willetts, 1971). Scanning electron microscopy of hyperparasitism of sclerotia of **Corticium solani** by **Aspergillus terreus** (Shigemitsu **et al**, 1978, 1981) showed that the conidiophores of the hyperparasites arose mostly from outer and a few from inner cortical layer of sclerotia.

The mycoparasite also invade the conidiophore/sporangiophores as detected in case of **Rhizopus nigricans, Cunnighamella echinulata** by **Fusarium udum** (Rai **et al**, 1978; Upadhyay **et al**, 1979). A necrotrophic mycoparasitic activity of **S. rolfsii** on **Aspergillus niger**, mainly on the conidiophore and **A. flavus** was also observed by Arora and Dwivedi (1979).

Many fungi produced volatile and non volatile metabolites, which have been found to inhibit the growth of several test pathogens. The metabolites may be either gaseous or residual form and counter acts with the substrates resulting inhibition of saprophytes growing in it. Several **Trichoderma** spp. are found to produce volatile and non-volatile metabolites inhibitory to the growth of other fungi. **T. viride** aggregates produce volatile inhibitor of characteristic smell, identified as acetaldehyde which inhibit the growth of **Fomes annosus** and **Rhizoctonia solani** (Dennis and Webster, 1971b).

Biological control of the soil-borne plant pathogens may be achieved temporarily or permanently through either

direct use of microorganism or by the stimulation of the antagonistic microorganisms in the soil. The most successful antagonist which controlled **S. rolfsii in vitro** and in nature is **Trichoderma harzianum**. Wells **et al** (1972) isolated **T. harzianum** from the diseased sclerotia of **S. rolfsii** and obtained 90% healthy plant in **T. harzianum** treated plots, compared to control. Backman and Rodriguez-Kabana (1975) also obtained a significant control of the disease by adding **T. harzianum** in the infested soil. They developed granular formulation of **T. harzianum** for easy applicability in the field without any residual effect. Stimulation of antagonists, germination-lysis phenomenon or mortality through release of volatile have been successfully achieved by modifying moisture regimes, organic amendments or use of non target pesticides.

Alternate wetting and drying as a means of biological control has been suggested. Such treatment leads to the release of nutrients from the sclerotia which enhance the growth of several antagonistic bacteria (Smith, 1972a,b). Nutrient release also results in significant loss of dry weight of sclerotia (Coley-Smith, 1979). Eruptive germination of sclerotia has also been induced by drying sclerotia (Smith, 1972a; Punja and Grogan, 1981a), exposing them to volatile compounds (alcohol and aldehyde) and to some extent sodium hypochlorite treatment (Linderman and Gilbert, 1973b; Beute and Rodriguez-Kabana, 1979a,b; Punja and Grogan, 1981a). These factors also enhance the nutrient leakage probably through the mobili-

zation of compartmentalized glucanases that act on stored glucans.

Seed inoculation with the mycelium of **T. harzianum** was found to control the wilt of lentil (Agrawal **et al**, 1977). The effectiveness was more when applied to the seed rather than to soil. Soil inoculation of the antagonist controlled the pathogen **S. rolfsii** causing diseases to different crop plants viz. root rot of sugar beet (Mathur and Sarbhoy, 1978), stem rot in lupin, tomato, ground nut, bean and egg plants (Chet **et al**, 1978; Elad **et al**, 1980). Dipping the cuttings of sugar cane in spore suspension of **T. lignorum** improved the seedling stand (Tokeshi **et al**, 1980).

Reduction of disease caused by **S. rolfsii** with mutual antagonism has been demonstrated in case of **S. rolfsii** with **Fusarium oxysporum** f. sp. **lycopersici** on tomato (Endo **et al**, 1976a). **Penicillium** sp. was also found to be successful as antagonist to **S. rolfsii in vitro** as well as in glass house, controlling the disease severity in tomato (Lozano and Lopez, 1977). In pre-fumigated soil, planting bean seed coated with hyperparasite **Penicillium** sp., protected the crop effectively in the glass house (Polanco and Castro, 1977).

Basu Choudhury and Gupta (1970) tried **Streptomyces nigrifaciens** against **S. rolfsii**, which was found to control

guar root rot. Partial control of **S. rolfsii** causing southern blight of **Cajanus cajan** with **Pseudomonas aeruginosa** and **Bacillus subtilis** was attributed to the production of an antibiotic called Pyocyanin (Brathwaite, 1978).

ENVIRONMENTAL FEATURE WITH GENERAL MATERIALS AND METHODS

Location of the Study Area

The present investigation was carried out in Shillong (Latitude 25.34°N and Longitude 91.56°E) the capital of Meghalaya, a hill state situated in the North Eastern Region of India. The altitude of the place varies from 1080m to 1990m with the highest peak lying in Shillong peak area.

Most of the experiments were conducted at Indian Council of Agricultural Research (ICAR), Experimental farm, Barapani (Fig.1), about 22 Km north of Shillong located at 25°30'30" latitude and 91°51'45" longitude. The climatic conditions are similar to that of Shillong except that it is slightly warmer and was found to be ideal place to conduct phytopathological experiments. Plate 1, showing the general view of ICAR farm, Barapani, with a site having soybean cultivation, where soil was found to be highly infested with the pathogen, **Sclerotium rolfsii.**

Vegetation

The vegetation of Shillong is characterised by evergreen tree species and can be broadly divided under:

(a) Subtropical pine forests.
(b) The rolling grassland.
(c) Mixed evergreen forest.
(d) Temperate forest.

Pinus kesiya, Royle ex Gordon, is the dominant tree species which occurs widely in Shillong along with other broad leaved angiosperms like **Schima wallichii** (DC) Korth.

Fig. 1: Map of Shillong and ICAR complex farm at Barapani showing different sites of experiments.

Shillong anc Barapani in Meghalaya

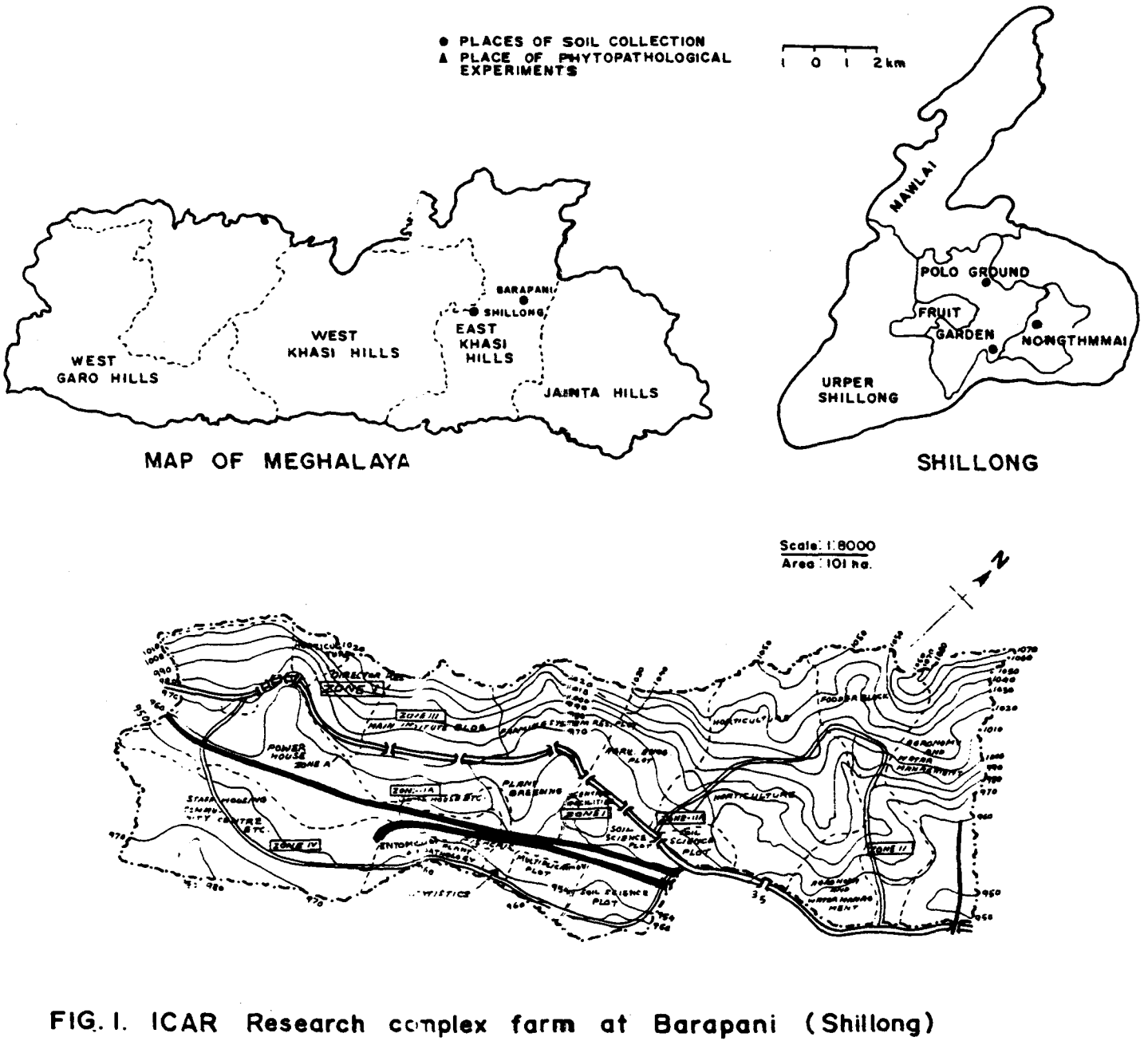


FIG. I. ICAR Research complex farm at Barapani (Shillong)

Plate 1: A portion of the site at Barapani ICAR farm showing soybean cultivation in **Sclerotium rolfsii** infested soil.

PLATE-I

S. khasiana Dyer., **Acacia dealbata** Link., **Rhododendron arboreum** Sm., **Alnus nepalensis** D. Don,, **Quercus** spp. etc. The common shrub or undershrub, species comprises of **Eupatorium adenophorum** Spreng., **E. riparium** Regel., **Artemisia parviflora** Roxb., **Lantana camara** Linn., **Duranta plumieri** Jacq. etc.

Soil types

The soil type of Shillong and around is mostly red laterite or brown loam soil. The soil in general is acidic in nature, and rich in nitrogen, but poor in potash and phosphorus content, which ranges between 20 Kg/acre to 50 Kg/acre (Zimba, 1978). In some areas sand content of the soil is found to be upto 90%.

Climate

The climate of Shillong is very much controlled by seasonal winds like that of other parts of the country. The South West monsoons and the North East winter winds influences climatic condition of Shillong.

The seasons may be divided into four groups basing on the data presented in Fig. 2.

a. Spring season - March and April.
b. Summer season - May to September
c. Autumn season - October and November
d. Winter season - December to February.

The spring season is characterised by the gradual

Fig. 2: The average monthly maximum and minimum temperature, morning and evening humidity and rainfall of Shillong for the period from January 1984 to December 1985.

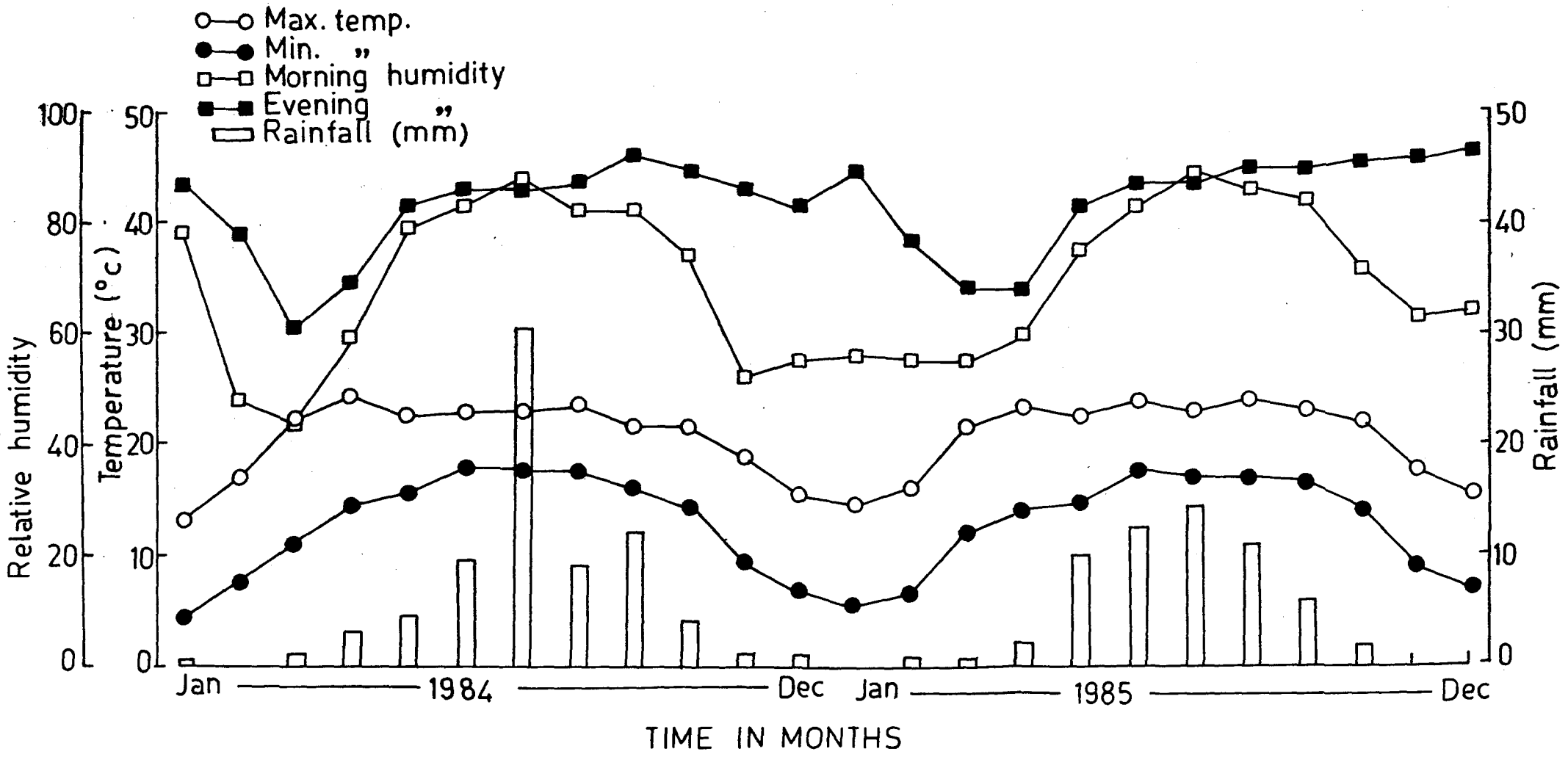

Max. temp.
Min. "
Morning humidity
Evening "
Rainfall (mm)
Relative humidity
Temperature (°c)
Rainfall (mm)
100
80
60
40
20
0
50
40
30
20
10
0
Jan
1984
Dec
Jan
1985
Dec
TIME IN MONTHS

FIG. 2

increase in temperature over the preceding winter months accompanied by occasional showers. Further increase in temperature gives rise to 'Summer' with heavy rainfall. A tremendous increase in temperature was recorded from middle of April to May. The average maximum temperature recorded during the study period (1984-1985) was24.7°c (during April, 1984) and minimum temperature was 4.5°c (January, 1984). The rainfall starts by the third week of May and continues upto the end of September and some times even upto mid of October. The highest average rainfall has been recorded in the month of July (1984) in a diurnal cycle. October and November are the two months when the climate is cool and temperature heralds the advents of autumn. This is followed by winter season lasting from December to February. During these months the climate is very cold with temperature lowered down to 4-5°c, with comparatively no rainfall. The lower temperature of winter, results into frost which can be seen sometimes early in the morning during December to January. Air remain humid mostly in the evening hours as observed from two years record (Fig.2).

Isolation of the pathogen

Infected soybean seedlings collected from the experimental field (i.e. ICAR farm, Barapani) were kept inside BOD incubator for 24 hours for the luxuriant growth of the pathogen i.e. **Sclerotium rolfsii.** The cottony mycelium from the infected portion of the soybean seedling was transferred

to PDA medium, which was then incubated at 25±1°c in dark (except where otherwise stated). The fungus grew within 3/4 days on the medium which was subsequently maintained as pure culture in PDA slants for further experiments.

Pathogenicity test

Resting structure (i.e. sclerotia) produced by the pathogen in culture, were thoroughly mixed with the top two inches of the sterile soil kept in pots (approx. 500 sclerotia/ pots) under glass house condition. Three weeks old test plants (i.e. soybean) grown in sterilised soil were gently transplanted to the artificially infested soil. A control was maintained in a similar manner without any sclerotium amendment to the soil. The pathogen was reisolated from the infected plants.

Determination of moisture content of the soil

Moisture content of the soil was calculated by taking 10 gm soil sample in a previously weighed crucible, and drying in an oven (105°c), overnight. The crucible was cooled in a desicator and reweighed, the difference of the weight gave the moisture content of the soil. The percentage of moisture content of different soil types were calculated as follows:

$$\text{Percentage of moisture} = \frac{A - B}{A - C} \times 100$$

Where, A = Fresh weight of soil together with crucible.
B = Dry weight of soil together with crucible.
C = Weight of empty crucible.

Soil pH

pH was determined by using soil and water suspension in the ratio 1:5 and was measured with an electronic digital pH meter.

Determination of water holding capacity of soil

This was done according to Piper (1944). Air dried soil was crushed in a porcelain mortar and sieved through a small sieve having pore size 0.5 mm in diameter. Whatman No.40 filter paper was placed on the brass box (5.6cm x 1.6cm) and fixed in position by means of a split brass spring. The weight of the box and filter paper was taken as the "weight of box unfilled" denoted as (A). Small portions of soil were then added to the box, which was gently tapped and the box was filled upto the top.

The box with soil was then placed in a petridish (9cm) and water was added to this dish to a depth of 1/4 inch. The box containing saturated soil was then removed and wiped to dryness and then the weight was taken. This "weight of box + saturated soil" has been denoted as (B). After weighing it was placed in an oven at 105°c and was dried for 24 hours until a constant weight was attained. It was cooled in a desicator and weighed again, recording the weight as "weight of box + oven dry soil" has been denoted as (C).

The amount of water absorbed by the filter paper was

determined as follows:

Five filter papers were weighed and saturated with water and then was placed on a flat glass plate and squeezed by gently rolling a glass rod over them. These were weighed again to determine the amount of water retained, from this the average amount retained by one filter paper denoted as (D) was determined. The water holding capacity was calculated as a percentage of the oven dried soil from the following expression -

$$\frac{B - C - D}{C - A} \times 100,$$

A, B, C, D having the value noted above.

Fungitoxicity In vitro

Fungal toxicity **in vitro** was determined by agar plate (poisoned food) assay. Each amendment/ chemical under test was calculated to give different concentrations (mentioned in respective chapters) were added to sterilised potato dextrose agar at 40±1°c, mixed thoroughly and poured in Petri dishes (9 cm diameter).

Mycelial disc (4 mm diameter) of the test pathogen (**S. rolfsii**), taken from the periphery of the vigorously growing cultures was placed in the centre of the amended agar plates. Assessment of the expansion of colony diameter from each disc on the Petri plates was done following incubation in the dark for 4 days at 25±1°c.

a. Method of fungal growth assessment: Growth of the pathogen on PDA was recorded by measuring the diameter of colonies in two directions at right angles and taking the mean of 3-5 replicates of each concentration of the chemicals.

b. Effect of amendments on the germination of sclerotia: Different concentrations of each amendments/chemicals were mixed thoroughly with the sterilised PDA medium kept at 40°±1°c and subsequently poured in the petri plates. Freshly cultured mature sclerotia (approx. 20/plate) was then inoculated in each plate containing cooled amended PDA medium and incubated for 3-5 days at 25±1°c in dark. Three such replicates for each concentration was prepared. Germination of sclerotia (%) was recorded after the above mentioned period of incubation.

Germination of sclerotia in the aqueous solution of the amendments/chemicals was observed as follows: Different concentrations of each amendment/chemical under test was prepared with ordinary tap water and was kept in three replicates. Twenty mature sclerotia was added to the test tubes containing different concentrations of the amendments/chemicals and was incubated for 7 days under natural condition. The percentage germination of sclerotia was recorded by direct observation and following the method of Agnihotri **et al** (1975).

Effect on soybean seed germination in vitro

The possible phytotoxic effect of different soil amend-

ments (organic and inorganic) and chemicals (antibiotics, nitrofurans and fungicides) on soybean seed germination (var. bragg) was investigated by keeping the seeds in the moist chamber made out of double layers of filter paper (Whatman No.40) in sterile plastic petri dishes. The seeds were surface sterilised with 0.1% mercuric chloride for 1 minute, washed repeatedly with sterile tap water. Aqueous solution of each amendment (different concentrations mentioned in the respective chapters) were prepared with distilled water and 5ml. of each solution was added per dish. Distilled water was added to serve as control. Twenty seeds were exposed to each treatment and was incubated at 25±1°c inside a BOD incubator. During the period of incubation, additional aqueous solution was added whenever necessary to avoid dryness. Germination percentage and radicle length of seedlings were recorded after 7 days of incubation.

Determination of phytotoxicity of different amendments/chemicals

This was done by dipping the roots of 4 weeks old soybean seedlings (for 48 hours) to the different concentrations of soil amendments/chemicals, prepared with Knop's normal solution. Visual phytotoxic symptom was recorded subsequently.

Effect of amendments (organic and inorganic) on the soil and rhizosphere microflora of soybean

After preliminary screening of the amendments (organic

and inorganic) on the soil and rhizosphere microflora of soybean grown in the non infested garden soil (not included in this thesis), few organic and inorganic amendments (as mentioned in respective chapters), were selected and used for the major experimentation in the pathogen infested soil, carried out at ICAR farm, Barapani.

Soil amendment of different concentrations were mixed thoroughly with infested soil in pots and was kept for 7 and 15 days for inorganic and organic amendments respectively, having 50% WHC for microbial decomposition under natural condition. Studies on the changes of soil microflora (quantitative and qualitative) due to amendments were done following a modified dilution plate method of Waksman (1932) and Timonin (1940) as described by Baruah and Dutta (1978). Peptone Dextrose Rose Bengal Agar (Martin, 1950), Starch Casein Agar (Kuster and William, 1964) and Erickson's Agar madium were used for fungi, actinomycetes and bacteria respectively.

10 gm of soil from each sample was taken in a 250ml conical flask containing sterile distilled water (100ml). This stock solution was thoroughly hand shaken for about 10-15 minutes. An initial 1:10 dilution was prepared and the subsequent dilutions were prepared by transferring 10ml of this suspension into 90ml sterile water, until the desired final dilutions were obtained, i.e. 1/10,000; 1/20,000 and 1/30,000 dilution were prepared for isolation for fungi, actinomycetes and bacteria respectively. Throughout the

investigation period same dilutions were used. 1.0 ml of each soil suspension (i.e. inoculum) from the above mentioned dilutions were transferred asceptically into petri dishes in triplicate and to that appropriate agar medium (cooled to 37°c) was added. The dishes were rotated gently by hand, so that the inoculum spreads uniformly. In each case seperate sterile pipettes were used for transferring the soil suspension. The plates were then incubated upside down at a temperature of 25±1°c in dark. The plates for fungi and actinomycetes were incubated for 5-7 days where as plates for bacteria were incubated for 24-48 hours. Total population per gram dry soil was calculated as follows:

$$\text{Total population} = \frac{\text{Total number of colonies X Inoculum X Dilution factor}}{\text{Dry weight of the soil/gram}}$$

For estimating the percentage of relative abundance of the particular genera/species of fungi, all the colonies of same genera/species were counted and calculated following the formula as follows:

$$\frac{\text{Total number of colonies of a particular genus/species}}{\text{Total number of colonies of all the genera/species}} \times 100$$

Different species of fungi was isolated and maintained in culture tubes containing agar slants of Czaspck-Dox agar medium and was preserved at 4°c. The identification of fungi was done with the help of the literature available (Gilman 1956; Barnett and Hunter, 1972 and Subramanian, 1971).

For actinomycetes and bacteria only total population

was considered and no attempt was made to identify upto the generic level. The same method of isolation of soil microflora was followed throughout the investigation period.

Rhizosphere Study

Soybean seedlings were grown from seeds in different amended soil and rhizosphere analysis of one month old seedlings were carried out following the procedures as described below:

Plants grown in amended soil or foliar sprayed plants (in case of antibiotics and nitrofurans) were picked up gently without disturbing the root system. The surplus soil was shaken from the roots and the soil adhering to the root system, was used. The root system with the rhizosphere soil, was cut off and 5 gm of the roots with adhering soil, was taken and placed in a conical flask containing 100 ml. sterile distilled water. The dilution in the flask was treated as 1:100 and was shaken for 5 minutes. Subsequently, different dilutions i.e. 1/10,000; 1/20,000 and 1/30,000 were made separately for fungi, actinomycete and bacteria respectively.

When the required dilutions were prepared the root systems were removed by means of forceps from the original flask. Water in the flask was then evaporated on a water bath and the soil residue was dried to a constant weight by taking it into a weighted crucible kept overnight in an

oven at 105°c. The dilution of the control soil was made from 10 gms of non-rhizosphere soil. The rhizosphere microflora estimated thus, from the soil dilution plates is based on the technique used by Waksman (1932) and Timonin (1940) and modified by Baruah and Dutta (1978). Peptone dextrose rose bengal agar, Starch casein agar and Erickson's agar media were employed for isolation of fungi, actinomycete and bacteria respectively. Pure cultures of these rhizosphere fungi were prepared in Czapeck dox agar slants for later identification.

The number of microorganisms developing on the plants were evaluated on the basis of oven dried rhizosphere soil. The ratio of microorganisms in the rhizosphere to those of non-rhizosphere (referred as control soil in the text and tables) expressed as 'rhizosphere effect' (RS/CS) was also calculated. The percentage of relative abundance of rhizosphere fungi was also calculated.

Survibality of Sclerotium of S. rolfsii in the amended soil (organic and inorganic materials/chemicals, antibiotics, nitrofurans and fungicides):

Soil amendments (i.e. organic and inorganic materials, antibiotics, nitrofurans and fungicides) at different concentrations (mentioned in the respective chapters) were mixed thoroughly with sandy loam garden soil in the plastic pots and was kept under natural condition for 15 days (in case

of organic materials) and 7 days (for inorganic chemicals) at 50% moisture level. The mouth of the pots were kept covered with perforated polyethylene sheet to avoid excessive evaporation. Four such replicates for each concentration were prepared. Freshly prepared mature sclerotia of **S. rolfsii** was mixed thoroughly (30 sclerotia/pot) with the amended soil at a depth of 5 cm and was incubated at room temperature for the requisite days. Sclerotia were recovered from each treated soil fortnightly, keeping the replicates undisturbed.

In case of antibiotics, nitrofurans and fungicides, the aqueous solution of different concentrations of these chemicals were added directly to the soil mixed with sclerotia.

Percentage of germinated sclerotia (i.e. survived) from the recovered sclerotia was assessed following the method of Agnihotri **et al** (1975) as described below:

Filter papers were immersed for two minutes in 1% glucose solution. The glucose impregnated filter papers (2 to 3) were then placed in the unsterilised petri dishes and the sclerotia were arranged over them. The plates were then covered and incubated at 25±1°c and germinability was observed visually after 2-3 days.

Estimation of test pathogen's (i.e. S. rolfsii) **population in amended soil**

Quantitative changes of **S. rolfsii** population in soil

due to amendment was carried out monthly, using a modified selective medium as proposed by Backman and Rodriguez-Kabana (1976). The composition of the medium has been described in page 48.

Assessment of disease severity

Seedling infection was first observed at the foot region when the plants were five weeks old. The number of plants showing disease symptom over total number per week was recorded and accordingly the percentage of infection was calculated.

Plant height

Height of soybean plant was measured from soil level to the top upto the apical bud, to observe the effect of soil amendment/chemical spray, on the growth and development of the plants.

Effect on yield

Yield/plant was determined by calculating the total weight of seeds (gm) from the plant of each set of experiments.

Method of cultural studies for biological control experiments

Inoculum of **S. rolfsii** for the biological control experiment and antagonistic fungi isolated from the soybean rhizosphere were grown in sterilised sand wheat medium separately. The medium was prepared as described below: The mixture of crushed wheat - one part and sand (thoroughly washed and

dried) nine part.

This was placed in the conical flasks (150 gm/500ml) and distilled water was added in sufficient quantity to moisten the medium. The medium was then sterilised at 17 lb pressure/sq.inch for 15 minutes, cooled and inoculated with **S. rolfsii** and other antagonistic fungi separately in asceptic condition. The flasks were then incubated at 25±1°c in dark for the growth of the test organisms.

Estimation of total sugars, amino acids and phenols in treated soybean radicles in vitro

Studies on the changes in total sugars, amino acids and phenols content in soybean radicles following organic and inorganic amendments were estimated using the methods of Peach and Tracey (1955) and Bray and Thrope (1954).

Estimation of total sugars (Peach and Tracey, 1955)

100 mg of powdered samples of separately treated soybean radicle, was crushed thoroughly in a mortar and pastle in 80% ethanol and was filtered through a Whatman No.1 filter paper. A slight milky white coloured filtrate was obtained in each case. This filtrate was treated with activated charcoal and centrifuged at 6000 rpm to get a clear supernatant. Ethanol was boiled off from the clear filtrate in a hot water bath and the volume was made upto 5 ml with the addition of double glass distilled water. 3ml of this solution was taken in

a test tube and 6 ml of Anthrone reagent (0.4% in H_2SO_4 w/v) was added gently holding the test tube in a cold water bath. Then the test tube was gently shaken and warmed in a boiled water bath for 3 minutes. A green colour was obtained. The transmittance of this coloured solution was observed in a spectrophotometer (Spectronic 20) at 610 nm. Standard curve was obtained from transmittance of varying concentrations of glucose solution treated with anthrone reagent as described for the samples. From this standard curve, the values of the total sugars of the samples were expressed in µg/100 mg dry weight of the sample.

Estimation of total amino acids (Peach and Tracey, 1955)

To the rest of the 2ml extract from above, 2.5 ml of acetate buffer (40g NaoH, dissolved in distilled water in a 100 ml volumetric flask, 100 ml of glacial acetic acid was added to it and the volume was made upto 1000 ml with distilled water) and 2.5 ml of ninhydrin solution (1% in isopropyl alcohol) was added simultaneously. The test tube containing the solution was placed in hot water bath for half an hour, a light purple colour was obtained. The transmittance of this coloured solution was observed in spectrophotometer at 540 nm. Standard curve was obtained from the transmittance of varying concentration of leucine solution treated with acetate buffer and ninhydrin solution as described for the sample. From the standard curve the value of the total

amino acids of the samples were expressed in µg/100mg dry weight of the sample.

Estimation of total phenols (Bray and Thrope, 1954)

100 mg of soybean radicle tissues were crushed separately in a mortar and pastle in 80% ethanol and filtered through a Whatman filter paper No.1. 1.0 ml of the extract was taken in a 25 ml graduated test tube and 1 ml of Folin ciocalteu reagent 1(N) and 2 ml of Na_2Co_3 solution was added. The tube was shaken and kept in a boiling water bath for 1 minute. The tube was cooled in a running tap water and the volume was made upto 25 ml with distilled water. The transmittance of this solution was observed in spectro photometer at 650nm. Standard curve was obtained from the transmittance of the varying concentration of Catechol, and the value was expressed as µg/100 mg of the sample.

Production of sclerotia of Sclerotium rolfsii

For **in vitro** studies related to the survival of sclerotia in soil, the fungus was grown on a crushed wheat medium.

The preparation of the medium was as follows:

10gm. of crushed wheat was taken in a 250ml conical flask. To this enough distilled water was added to moisten the medium. This was then sterilised at 17 lb pressure/sq.inch for 15 minutes. Sclerotia of **S. rolfsii** was then inoculated when cooled and incubated at 25±1°c in dark.

Composition of the medium used for different experiments

Potato Dextrose Agar (PDA) medium (Riker and Riker, 1936)

Cut potato cubes were weighted out and boiled in water until soft and squeezed to get the pulp through a fine sieve and boiled until dissolved. The extract was used to make PDA according to the following formula.

	g/litre distilled water
Potato (Peeled)	200.00
Dextrose	20.00
Calcium carbonate	0.20
Magnesium sulphate	0.20
Bacto-agar (Difco)	20.00
Streptomycin	0.25

Streptomycin was added when the medium was cooled to 45°c.

Water Agar medium

Bacto-agar (Difco)	15 gm
Distilled water	1000 ml

Czapeck Dox Agar medium (Raper and Thom, 1949)

	g/litre distilled water
Sodium nitrate	2.00
Dipotassium hydrogen phosphate	1.00
Magnesium sulphate	0.50
Potassium chloride	0.50
Ferrous sulphate	0.01
Sucrose	30.00
Bacto-agar (Difco)	15.00

Czapeck Dox liquid medium

The formula is as before with the exception of bacto-agar, which is omitted.

Peptone-Dextrose Rose Bengal Agar medium (Martin, 1950)

	g/litre distilled water
Dextrose	10.00
Peptone	5.00
Potassium dihydrogen orthophosphate	1.00
Magnesium sulphate	0.50
Rose Bengal (1%)	3.00 ml
Bacto-agar (Difco)	20.00
Streptomycin sulphate	30.00 mg

Streptomycin sulphate was added when the medium was cooled at 45°c.

Starch Casein Agar medium (Kuster and Williams, 1964)

	g/litre distilled water
Starch	10.00
Casein	0.30
Potassium nitrate	2.00
Sodium chloride	2.00
Potassium hydrogen orthophosphate	2.00
Magnesium sulphate	0.05
Calcium carbonate	0.02
Ferrous sulphate	0.01
Bacto-agar (Difco)	10.00

Erickson's Agar medium

	g/litre distilled water
Calcium nitrate	0.33
Calcium sulphate	0.80
Magnesium sulphate	0.70
Potassium sulphate	0.025
Dipotassium hydrogen phosphate	0.005
Sodium bicarbonate	0.02
Glucose	0.01
Yeast extract	0.005
Ferric chloride	Trace
Bacto-agar (Difco)	15.00

All the above mentioned media were sterilised at 120°c at 17 lb pressure/sq.inch for 15 minutes in conical flasks (Corning) having air tight cotton plugs at the mouth.

Selective medium for isolation of Sclerotium rolfsii from soil (Backman and Rodriguez-Kabana, 1976)

I.	Potassium dihydrogen orthophosphate	1.0 gm
	Magnesium sulphate	0.5 gm
	Potassium nitrate	2.0 gm
	Thiamine hydrochloride	1.0 mg
	Minor element solution	10.0 ml
	Gallic acid	160.0 mg
	Potassium oxalate	10.0 gm
	Glucose*	2.0 gm
	Distilled water	250 ml

These were thoroughly dissolved in distilled H_2O, filtered and sterilised, the pH was adjusted to 4.2 with HCl.

*Glucose was added as an additional chemical for better isolation of the **S. rolfsii** from soil.

II.	Agar	20 gm
	Distilled water	750 ml

Steam sterilised for 15 minutes at 121°c, cooled to 60°c.

III. I with II was combined and poured in the plates containing inoculum (1 ml) immediately. The plates were then incubated in dark (25±1°c).

Preparation of Minor element solution

A stock solution containing the following chemicals was prepared.

Ferrous sulphate	1.0 gm
Zinc sulphate	1.0 gm
Manganous sulphate	0.6 gm
Distilled water	1000 ml

Formula of Knop's normal culture solution

	g/liter distilled water
Potassium nitrate	1.0
Acid Potassium phosphate	1.0
Magnesium sulphate	1.0
Calcium nitrate	4.0
Ferric chloride	a few drops

This is a stock solution of 0.7% strength, for water culture, six litres of water was added to this stock solution for experimental work.

PART I SOIL SURVIVAL

CHAPTER I

SENSITIVITY OF SCLEROTIUM ROLFSII TO THE SEASONAL VARIATION OF SOIL FUNGISTASIS IN RELATION TO THE PHYSICO-CHEMICAL FACTORS, MICROBIAL POPULATION, AND ACTIVITY IN DIFFERENT SOIL TYPES

INTRODUCTION

Soil microorganisms play a very important role in root diseases both in forest plants and in agricultural crops. The Propagules such as spores, thick walled chlamydospores, sclerotium, resting hyphae of soil-borne plant pathogens remain dormant in soil due to fungistasis, a natural inhibitory factor present in all types of soil supporting microbial growth (Dobbs and Hinson, 1953; Dobbs and Bywater, 1959). This inhibitory factor is absent in the region of less microbial growth i.e. in highly acidic soil, deep subsoil and in sterilised soil, strongly supports its biological origin (Dobbs and Hinson, 1953; Dobbs and Bywater, 1959; Lockwood and Lingappa, 1963; Dutta **et al**, 1982).

Microbial production of volatile unsaturated hydrocarbons such as ethylene has been correlated to fungistasis, as the top soil released greater amount, which was found to be more fungistatic compared to subsoils producing lesser amount of ethylene (Dutta **et al**, 1982). Ethylene, the main inhibitory substance that produced in soils as a result of microbial activity (Smith and Restall, 1971; Smith, 1973) and the concentrations varying upto 75 parts $/10^6$ (in water logged soil) have been reported (Smith and Dowdell, 1974) causing soil fungistasis. A wide range of fungi (Ilag and curtis, 1968) and soil bacteria (Chen **et al**, 1976; Primrose and Dilworth, 1976) with the ability to form ethylene in pure culture have been isolated and identified. It has been

suggested that spore forming anaerobic bacteria (Smith and Cook, 1974) and the fungus **Mucor hiemalis** (Lynch, 1975) constitute the most important groups of ethylene forming microorganisms in soil. There could be an interaction between nutrient levels for microbial growth and the concentration of ethylene required to maintain fungistasis in soil (Smith, 1973).

The sclerotia of **Sclerotium delphinii** and **S. rolfsii** have been shown to be particularly affected by drying and wetting treatments (Coley-Smith **et al**, 1974) resulting in the leakage of endogenous nutrients, stimulating the growth of soil microorganisms, which colonize and invade the sclerotia (Gilbert and Linderman, 1971).

Nutrient independent propagules e.g. Sclerotia of **Sclerotium rolfsii** and conidia of **Fusarium** spp. whose germination is suppressed partially by the nutrient stress or exudation imposed by competing soil microflora (Bristow and Lockwood, 1975; Sneh and Lockwood, 1976; Filonow and Lockwood, 1979). Soil microflora rapidly utilizes the energy yielding substrates, including germination promoting nutrients (i.e. exudates), released from the fungal propagules to environment due to stress has been termed as microbial 'nutrient sink' resulting in fungistasis (Lockwood, 1977, 1981).

Very little information is available on the dehydrogenase activity which measures the net effect of numerous

enzymatic processes taking place in the soil. It is regarded as being more dependent on the general metabolic status of the soil microflora than the activity by specific cell free enzyme activity upon certain substrates.

Lenhard (1956) for the first time used 2, 3, 5 triphenyl tetrazolium chloride (TTC) in the studies of microbial activity in soil. The method is based on the assumption that in the absence of oxygen, TTC acts quantitatively as the terminal H^+-aceptor for dehydrogenase system with the formation of red triphenyl tetrazolium formazan (TTF).

$$TTC + 2H^+ + 2e^- = 2TTF + Hcl.$$

In the present investigation, an attempt was made to determine the sensitivity of **S. rolfsii** to the seasonal variation of soil fungistasis together with other soil (i.e. soil pH, moisture, organic matter, sugar and enzyme activity) and environmental (i.e. rainfall, temperature and humidity) factors.

MATERIALS AND METHODS

Sclerotium rolfsii Sacc. isolated from the infected soybean (**Glycine max** L.) and **Fusarium solani** (Mart.) APP. WR. Synder and Hensen isolated from **Albizia labbek** (Linn.) Benth., were used as test organisms and were maintained in Potato dextrose agar medium.

Three soil types viz. forest, grassland and garden

from three different sites were collected at random from five different spots, for each type of soil. Samples for each site were mixed thoroughly, air dried and sieved (through 2mm pore size). 40-60 grams of each soil sample was taken separately in petridishes (20 cm diameter) and were brought to 60% water holding capacity by adding distilled water and different experiments were performed following the methods described below:

i) **Sensitivity of Sclerotium to soil fungistasis**

Soil samples prepared as described above were kept in two sets., One set of each soil type was used as natural, whereas the other set was sterilised by autoclaving (at 17lb/sq inch Pressure for 15 mins). Triplicate samples were prepared for each set. Twenty mature sclerotia of **S. rolfsii** were inserted into large petridishes (20 cm) containing experimental soil and was incubated for 48-72 hours at 25±1°c. Percentage of germination was calculated after the said incubation period.

ii) **Determination of Physical Properties of the soil samples**

Moisture content: It was measured by drying known amount of soil samples in an oven at 105°c (as described in page 32).

Soil pH: Soil pH was determined with the help of an electronic digital pH meter as described earlier (page 33).

Organic matter content: Thiswas determined by rapid

titration method of walkley and Black (1934). Two grams of dried and sieved (through 0.2 mm mesh) powdered soil was taken in 500 ml conical flask containing 10 ml of 1(N) $K_2Cr_2O_7$ solution and was mixed thoroughly. Subsequently, 20ml of concentrated H_2SO_4 was added and shaken gently. This was then allowed to stand for 20 minutes. 200 ml of H_2O and 10 ml of 85% Phosphoric acid were added to the flask. Few drops of diphenylamine was used as an indicator and was titrated against ferrous sulphate 1(N) solution. Calculations were done following the formula:

$$\% \text{ Organic matter} = 10 \left(1 - \frac{T}{S}\right) \times 1.34$$

Where S = Blank titration
T = Sample titration.

Water holding capacity of the soil: This is determined following the procedure as described earlier (page 33).

iii) **Estimation of total microbial Population in soil**

Seasonal variation in microbial population was determined by a modified soil dilution plate method as described by Baruah and Dutta (1978) (described in page 37). Martin's rose bengal agar (Martin, 1950); Starch casein agar (Kuster and Williams, 1964) and Erickson's agar medium was employed for isolating fungi, actinomycete and bacteria respectively. The percentage of relative abundance of different fungal species was also calculated using the formula described earlier (page 38).

iv) **Estimation of total sugars**

Estimation of total sugars in both natural and in sterilised soil samples were carried out following the method described by Peach and Tracey (1955), as detailed in page 43 .

v) **Enzyme assay**

The enzymatic activity (dehydrogenase) of the soil microbes was assayed by a modified method as described by Casida **et al** (1964). Replicated surface layer (10 cm deep) soil samples from different sites were collected at random in monthly intervals. Soil samples collected from each site (i.e. forest, garden, grassland) were then mixed separately to make a composite sample of each soil type. The samples were processed further on the same day (within 3-4 hours) to determine the dehydrogenase activity.

Dehydrogenase activity of soil was determined with 2, 3, 5 triphenyl tetrazolium Chloride (TTC) reduction technique. Each soil sample was weighed (5 gm/tube) into three sterile screw cap tubes. To each tube 0.1 gm $CaCO_3$ and 1.0 ml of 1.5% (w/v) 2, 3, 5-triphenyl tetrazolium chloride (TTC) was added. To this, 4 ml of distilled water was added which was found to be enough to saturate the soil and form a liquid layer on the soil samples. This ensures adequate anaerobiosis for TTC reduction. The content of each tube was mixed thoroughly and then incubated at 37°c for 24 hours. The extraction of triphenyl tetrazolium formazan produced was carried out with

methanol. The soil suspension was subsequently passed through the Whatman No. 40 filter paper by shaking with methanol and washed down into a 50 ml volumetric flask, until no more colour could be extracted and the volume was made upto the mark by adding methanol. During this extraction procedure it was necessary to keep the sample wet at all the time to avoid the air drawn through the soil. The transmittance of the pink coloured solution was observed in spectro calorimeter at 485 nm, using methanol extract (without soil) for the control as blank.

vi) **Sporostatic effect of ethylene and its possible role in soil fungistasis**

Experimentation with ethylene (in gaseous form) **in vitro** is hazardous, as it is neither absorbed nor adsorbed by the soil and is difficult to handle. Therefore, a chemical under the trade name of Ethrel (2 chloroethane phosphonic acid) which is a most effective ethylene generator (40-90% in aqueous solution at pH4) (Warner and Leopold, 1969) has been used in the present work to determine the effect of ethylene on soil microorganisms and to determine its role in soil fungistasis.

a) Sensitivity of **sclerotium** of **S. rolfsii** to ethylene:

Soil collected from three different sites (as described earlier) were air dried, sieved (through 2mm mesh sieve) and sterilised by autoclaving (at 17 lb/Sq inch pressure for 15 mins). Ethrel (a.i.39.2% w/w) at different concentrations

in aqueous solution (1,10,50,100 µl/L), was added directly to soil to saturation point.

The promoter (i.e. nutrient) and inhibitor (i.e. ethylene) theory of Smith (1973) was examined by an experiment performed with ethrel and glucose (1 and 10%) by adding to soil at different concentrations. Twenty mature sclerotia of **S. rolfsii** were placed on the surface of each soil sample and was incubated for 48-72 hours at 25±1°c. A control was also made using sterilised soil and was incubated in the same manner. Percentage germination of sclerotia was recorded after the incubation period. The experiment was performed in the month of December, when the fungistatic effect in the soil was found to be the least.

b) Sensitivity of conidia of **F. solani** to ethylene:

(i) In Soil: Test Pathogen **Fusarium solani**, was used in this experiment to compare its sensitivity to soil fungistasis with **S. rolfsii**. The soil (garden soil only) was prepared as described earlier and a modified agar slide method (Dutta and Isaac, 1979) was used for assaying the fungistatic effect in soil. Spore suspension ($2x10^6$ spores/ml) was prepared from 8-10 days old culture of the test fungi by flooding the culture with sterilised distilled water. The spore suspension was then mixed with 2% molten agar kept at 40°c. Since agar may be a source of nutrient essential for fungal spore germination and thus mask the fungistatic activity (Romaine

and Baker, 1973), the agar was washed several times. Sterilised glass slides were then dipped into 2% spore seeded agar solution. Filter papers were cut into the size of glass slides and each spore seeded agar slide was covered with a piece of filter paper to avoid the soil particles attached on the agar which otherwise makes the microscopic observation difficult. After complete solidification of the agar, the spore seeded agar slides were buried into sterile petridishes (20cm diameter) containing sterilised garden soil and was drenched with ethrel and ethrel+glucose (1 and 10%) in different concentrations (1,10,50,100 µl/L). The slides so treated were kept for incubation at 25±1°c for 24 hours. After incubation, the soil from the spore seeded agar slide was rinsed away and the slides were examined under microscope. The percentage of spore germination and germ tube length (µm) was calculated. A control was maintained by placing spore seeded agar slide in sterilised soil drenched with distilled water only. The percentage of inhibition was calculated according to Isaac and Heale (1961).

(ii) A study on the effect of ethrel on spore germination and germ tube growth of **F. solani** in aqueous solution was also made. In this case, a drop of spore suspension (of known strength) and a drop of aqueous solution of ethrel (known concentration) was taken on a cover slide and inverted over a sterilised cavity slide, in such a way that it must hang over the cavity. The moist atmosphere was maintained

using a drop of ethrel solution (of same concentration) in that cavity. A control was prepared taking a drop of spore suspension and a drop of distilled water only. The spore germination and germ tube length was recorded after 24 hours of incubation at 25±1°c and their percentage of inhibition was calculated according to Isaac and Heale (1961). Similar experiments were also made with ethrel mixed with glucose (1 & 10%) at different concentrations.

vii) **Effect of ethylene on soil microbes**

Different concentrations of ethrel (1,10,50,100 µl/L) in aqueous solution was added to the soil (garden soil) in sterile glass jar upto the saturation point. The lid of the jar was kept air tight and was incubated for 10 days under laboratory condition. A control set was also prepared in the same manner using tap water instead of aqueous solution of ethrel. The total microbial population (i.e. fungi, actinomycete and bacteria) in treated and control soil was evaluated following the modified soil dilution plate method as described by Baruah and Dutta (1978).

viii) **Soil fungistasis in relation to organic and inorganic soil amendments**

Organic (i.e. green leaf of **Eupatorium adenophorum, E. riparium, Pinus kesiya, Helianthus annuus** and dried powdered poultry litter) and inorganic (i.e. calcium carbonate, calcium

nitrate, rock phosphate, zinc sulphate, urea and ammonium nitrate) amendments were added to sandy loam garden soil in different proportions (1, 2, 3 and 0.1, 0.25, 0.5% w/w for organic and inorganic respectively), and was kept for 15 and 7 days respectively under natural condition . Moisture level of the soil was adjusted to 50% WHC. The organic and inorganic amended soil as mentioned above were then taken seperately and was placed in petridishes (in triplicate), 7-8 days old sclerotia (20 numbers/plate) was placed on the surface soil and was incubated at 25±1°c percentage of inhibition in the germination of sclerotium was calculated following the incubation period.

RESULTS

i) Sensitivity of Sclerotium to soil fungistatsis

From Fig. 3.1, it was evident that fungistasis is a seasonally variable soil factor present in all the three types of soil tested. Inhibition in germination of sclerotium in soil, was higher in the month of July in case of forest, April and July in case of garden and May and June in case grassland soil. This inhibition in germination decreased with the seasonal decrease of temperature (i.e. in winter). Highest percentage of sclerotial germination was observed in the month of January in case of grassland and February in case of forest and garden soil. The seasonal variation of soil fungistasis seemed to have a relationship with the

Fig.3.1 Seasonal variation of soil fungistasis in relation to environmental condition.

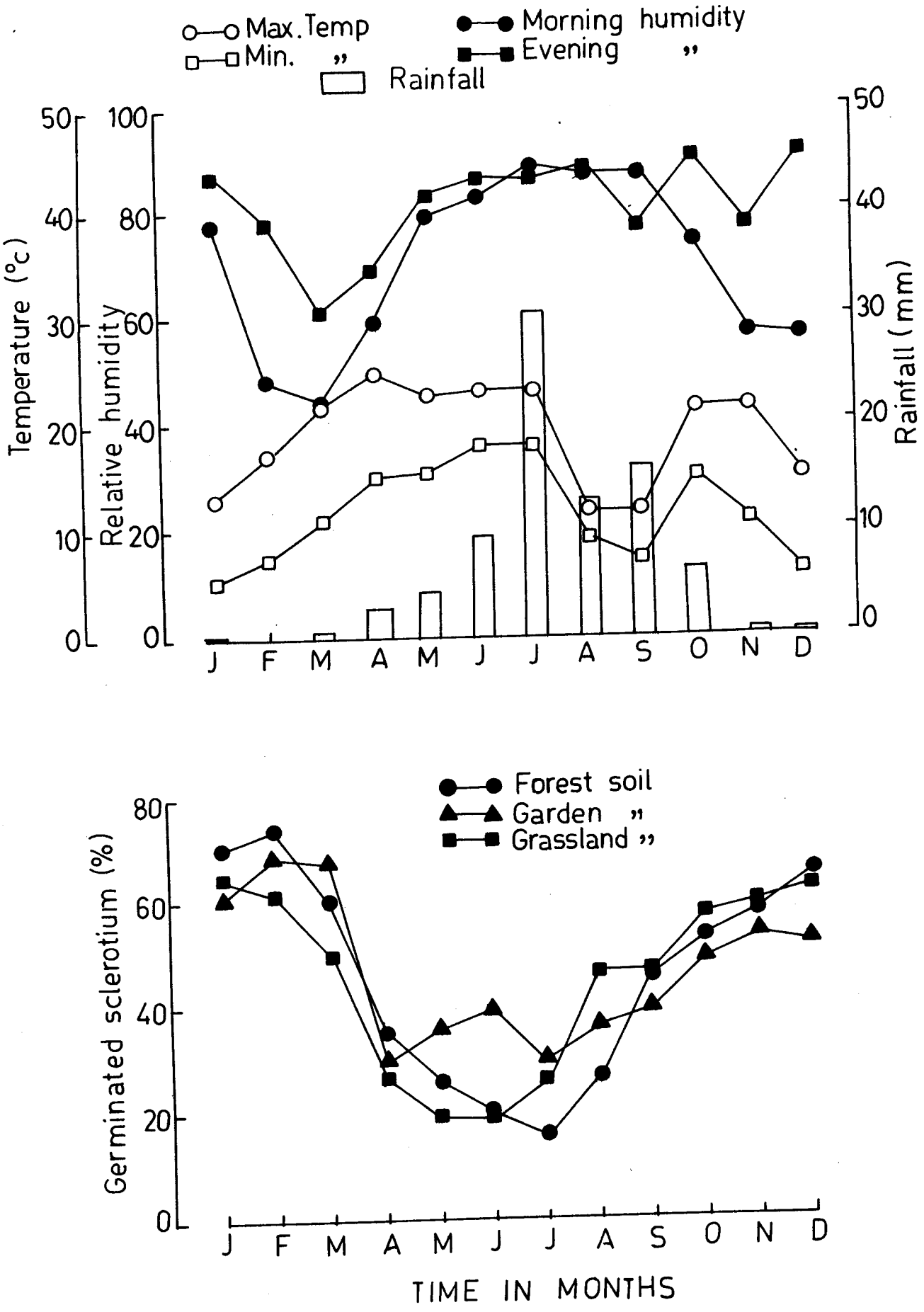

Max. Temp
Min. "
Morning humidity
Evening "
Rainfall
Temperature (°c)
Relative humidity
Rainfall (mm)
50
40
30
20
10
0
100
80
60
40
20
0
J F M A M J J A S O N D
Forest soil
Garden "
Grassland "
Germinated sclerotium (%)
80
60
40
20
0
J F M A M J J A S O N D
TIME IN MONTHS

FIG.3·1

environmental condition (i.e. temperature, humidity, rainfall). (Fig. 3.1)

The inhibition in germination of sclerotia was found to be less in sterilised soil, compared to natural soil (non sterilised). In most of the cases, 100% germination of sclerotium was observed but a slight inhibition (i.e. 5-13.3%; 3.30-20% and 1.7-13.3%) was observed in case of forest, garden and grassland soil respectively (Table 1.1). The mycelium developed from the germinated sclerotium always grew well in sterilised soil compared to non-sterilised soil (Table.1.1)

ii) **Physico-chemical properties of soil**

Moisture content (range varies from 15-31; 14-30 and 15-27 in case of forest, grassland and garden soil respectively) and pH (4.50-5.95; 4.84-6.54 and 4.90-6.10 in case of forest, grassland and garden soil) of the soil was found to be seasonally variable. Organic matter content (%) of different soil types also varied. Higher accumulation was observed in case of forest (5.60-8.65) followed by garden (2.50-5.10) and grassland (1.10-4.87) soil (Table 1.2).

iii) **Total microbial population in soil**

It was observed that the actinomycete population increased from July to November with a maximum increase in the month of September and October in all the soil types tested (Fig. 3.2 B). Fig. 3.2C showed an increase in bacterial

TABLE 1.1 : Seasonal variation in Sclerotial germination and mycelial growth of **S. rolfsii** in different soil types.

Months	Forest Soil				Garden Soil				Grassland Soil			
	Percentage inhibition in Sclerotial germination		Radial growth of mycelium of the germinated Sclerotium(mm) (Mean ±S.E.)		Percentage inhibition in sclerotial germination		Radial growth of mycelium of the germinated Sclerotium(mm) (Mean ±S.E.)		Percentage inhibition in Sclerotial germination		Radial growth of mycelium of the germinated Sclerotium(mm) (Mean ± S.E.)	
	A	B	A	B	A	B	A	B	A	B	A	B
January	5.00	30.00	27.00 ±0.95	24.00 ±1.19	Nil	38.30	31.00 ±1.76	28.00 ±0.68	5.00	35.00	22.00 ±1.16	23.00 ±1.38
February	10.00	26.70	30.00 ±3.29	30.00 ±1.76	Nil	30.00	39.00 ±1.74	24.00 ±0.99	1.70	38.30	23.00 ±1.38	19.00 ±1.74
March	10.00	40.00	15.50 ±2.69	10.00 ±1.20	Nil	31.70	25.60 ±3.47	20.00 ±1.49	10.00	50.00	13.40 ±2.85	15.00 ±1.92
April	Nil	65.00	18.50 ±2.60	14.40 ±1.61	Nil	70.00	46.40 ±1.60	13.00 ±2.24	Nil	73.30	35.00 ±7.30	14.20 ±3.28
May	10.00	73.30	20.00 ±1.44	5.00 ±0.86	20.00	63.30	32.00 ±1.67	20.00 ±1.25	10.00	80.00	30.00 ±4.05	15.00 ±1.70
June	13.30	80.00	25.00 ±2.05	2.00 ±0.00	13.30	60.00	25.30 ±2.15	29.80 ±0.86	Nil	80.00	24.00 ±3.66	9.00 ±2.55
July	Nil	83.30	15.00 ±1.45	5.00 ±0.60	Nil	70.00	23.00 ±1.19	20.00 ±1.86	5.00	73.30	30.00 ±3.65	25.00 ±2.99
August	Nil	73.30	12.20 ±1.98	8.30 ±2.73	13.30	63.30	13.20 ±2.34	18.00 ±0.68	13.30	53.30	14.80 ±2.49	12.80 ±3.94
September	Nil	53.30	21.00 ±2.10	14.80 ±3.05	Nil	60.00	33.90 ±6.08	21.80 ±1.77	6.70	53.30	25.40 ±3.13	11.00 ±2.95
October	6.70	46.70	22.40 ±3.76	13.00 ±7.49	Nil	50.00	25.00 ±3.20	18.00 ±1.84	Nil	41.70	20.80 ±5.76	11.00 ±0.82
November	Nil	41.70	20.00 ±1.12	15.00 ±1.40	3.30	45.00	26.00 ±2.23	20.00 ±1.39	Nil	40.00	20.00 ±1.32	16.00 ±2.10
December	Nil	35.00	24.00 ±1.60	19.00 ±1.67	Nil	46.70	30.00 ±2.03	26.00 ±1.76	Nil	36.70	23.00 ±1.23	20.00 ±1.34

A. Sterilised soil
B. Natural Soil

Calculation based on 60 sclerotia in each case.

TABLE 1.2 : Seasonal variation in Physico-chemical properties of three soil types tested.

Soil types	Parameters*	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Forest Soil	Moisture Content(%)	16.00	18.00	20.00	22.00	25.00	31.00	28.00	25.00	20.00	17.00	15.00	20.00
	Organic matter Content(%)	7.50	6.90	5.60	5.85	6.20	6.45	6.90	7.38	6.00	8.00	8.52	8.65
	pH	5.10	4.50	5.21	5.95	5.62	5.10	5.30	5.20	5.70	4.57	5.60	5.69
Garden soil	Moisture Content(%)	17.00	20.00	22.00	18.00	20.00	27.00	25.00	15.00	19.00	15.00	15.00	20.00
	Organic matter Content(%)	4.00	3.20	4.50	3.65	3.00	2.50	3.70	3.00	4.20	3.80	5.10	3.67
	pH	6.10	5.90	5.00	4.90	5.19	5.26	5.30	5.69	5.96	5.94	5.17	5.80
Grassland soil	Moisture Content(%)	14.00	19.00	20.00	22.00	20.00	30.00	28.00	15.00	18.00	20.00	18.00	17.00
	Organic matter Content(%)	1.10	1.60	3.20	1.45	1.90	2.00	2.70	2.50	2.00	4.02	4.87	3.08
	pH	6.10	5.90	4.85	5.10	5.42	5.30	4.84	5.76	4.97	6.54	5.65	5.00

*Mean of three replicates in each case.

Fig.3.2 Seasonal variation of soil microbial population (where, A=fungi; B=actinomycete; C=bacteria) in three different soil types.

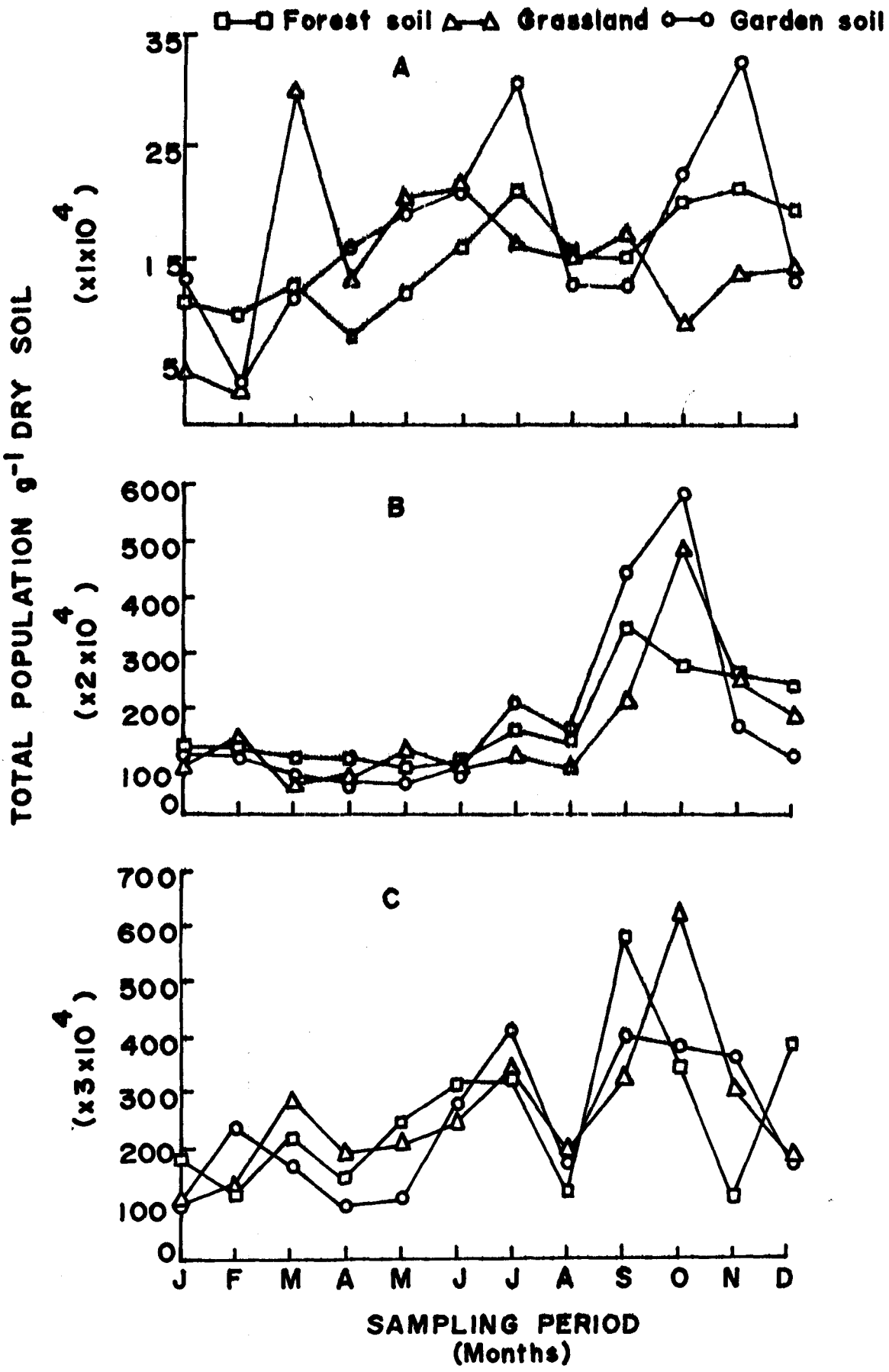

Forest soil
Grassland
Garden soil
A
B
C
TOTAL POPULATION g⁻¹ DRY SOIL
(x1x10⁴)
(x2x10⁴)
(x3x10⁴)
35
25
15
5
600
500
400
300
200
100
0
700
600
500
400
300
200
100
0
J F M A M J J A S O N D
SAMPLING PERIOD
(Months)

FIG. 3·2

population during July (garden), September (forest) and October (grassland), while higher population of fungi was recorded in the month of June-July (Fig. 3.2A). In general, seasonal fluctuation of microbial population was observed in all the cases.

It was evident from Fig. 3.3 that the **penicillium** spp. are the dominant genera present in all the soil types tested. Higher occurrence of **penicillium** (above 50%) was observed in the month of April, August and December in case of forest, whereas in garden soil, the increase was in the month of May, July, September and December. In grassland soil, higher **penicillium** spp. population (45%) was observed in the month of September. Compared to other soil types, grassland was found to have poor organic matter content and harbour lesser microbial population.

Other major groups of dominant fungi were **Aspergilli**. **Fusarium** spp., Mucorales and **Trichoderma** spp. Their total population was also found to be seasonally variable. About 36 species belonging to 14 genera and some unidentified genera of soil fungi were isolated from three different soil types also show a seasonal variation in their relative abundance in the soil (List of fungi presented in table 1.3).

iv) **Estimation of total sugars in soil**

Sugar concentration in all the soils were found to

Fig.3.3 Seasonal variation of some dominant genera of fungi in varied soil types.

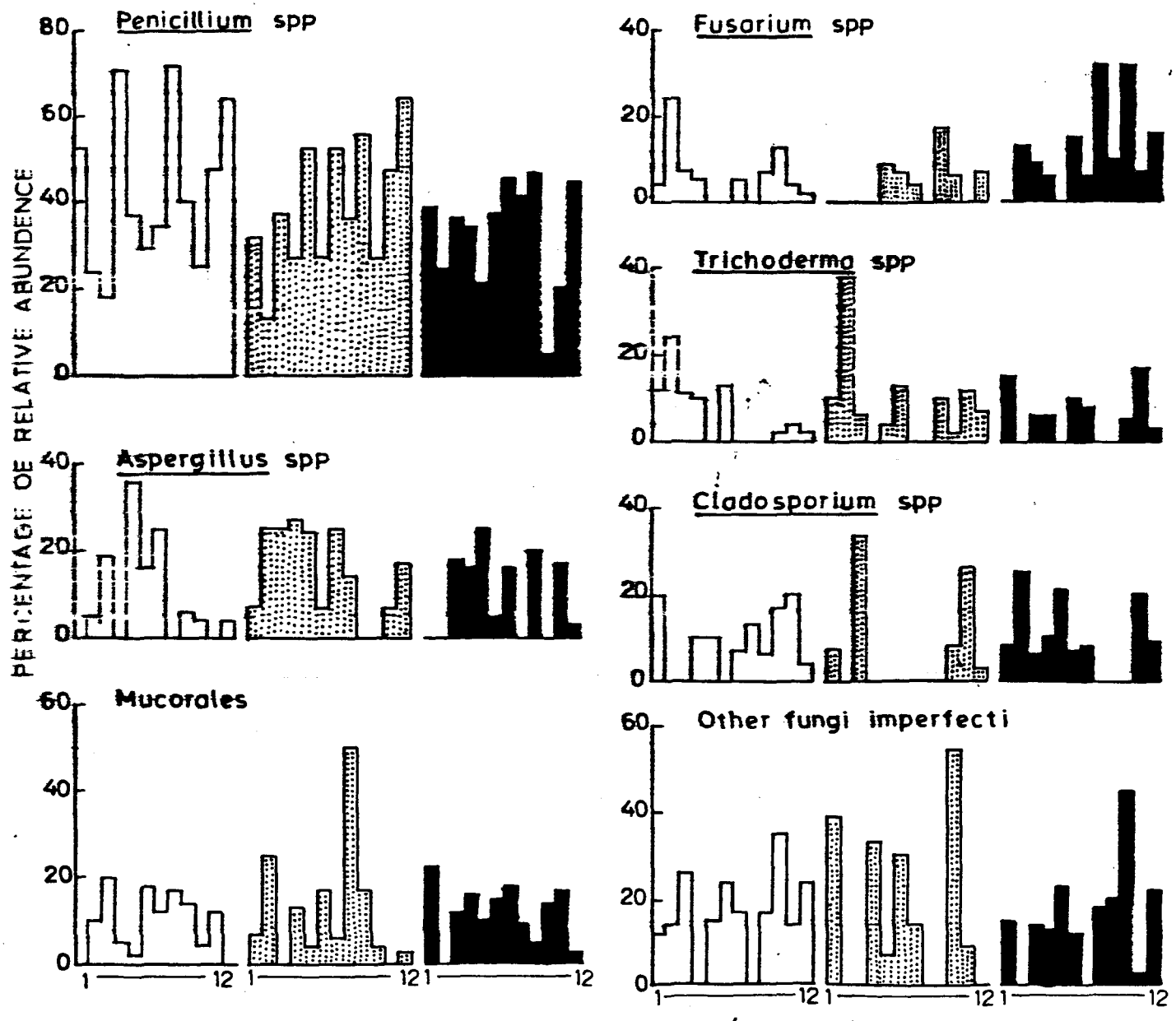

Penicillium spp
Fusarium spp
Trichoderma spp
Aspergillus spp
Cladosporium spp
Mucorales
Other fungi imperfecti
PERCENTAGE OE RELATIVE ABUNDENCE
SAMPLING PERIOD (MONTHS)

FIG.3·3

be seasonally variable. It has been observed that the amount of sugar is always high in sterilised soil compared to the natural soil (Fig. 3.4). Winter months (i.e. November to January) showed higher accumulation of sugars, both in natural and sterilised soil. A comparative higher amount of sugars (185 and 155 µg/100mg), was observed in forest soil (sterilised and natural) in the month of December, whereas, its amount reduced considerably during the month of June (Fig. 3.4A). Similar results were also observed in garden and grassland soil, where the highest accumulation of sugar was observed in September and December respectively (Fig. 3.4B,C).

v) **Enzyme assay**

It can be seen from Fig. 3.5 that dehydrogenase activity was always higher in forest soil compared to grassland and garden soil. This activity increased gradually (January to June) in forest and grassland soil but there was a fluctuation in the activity in garden soil.

vi) **Sporostatic effect of ethylene and its possible role in soil fungistasis**

a) Sensitivity of Sclerotium to ethylene:

Ethrel (2 Chloro-ethane phosphonic acid) was found to induce fungistatic effect in sterilised soil, which was otherwise nonfungistatic. The percentage germination of sclerotium in ethrel (100 µl/L) amended forest, garden and grassland soil was 12.5%, 18.75% and 13.5% respectively. The inhibi-

Fig.3.4 Seasonal variation of total sugars present in different soil types, viz. A=Forest soil, B=Garden soil, C=Grassland soil.

[The complete line indicates total sugars in sterilised soil, while broken line indicate total sugars in non sterile (natural) soil.]

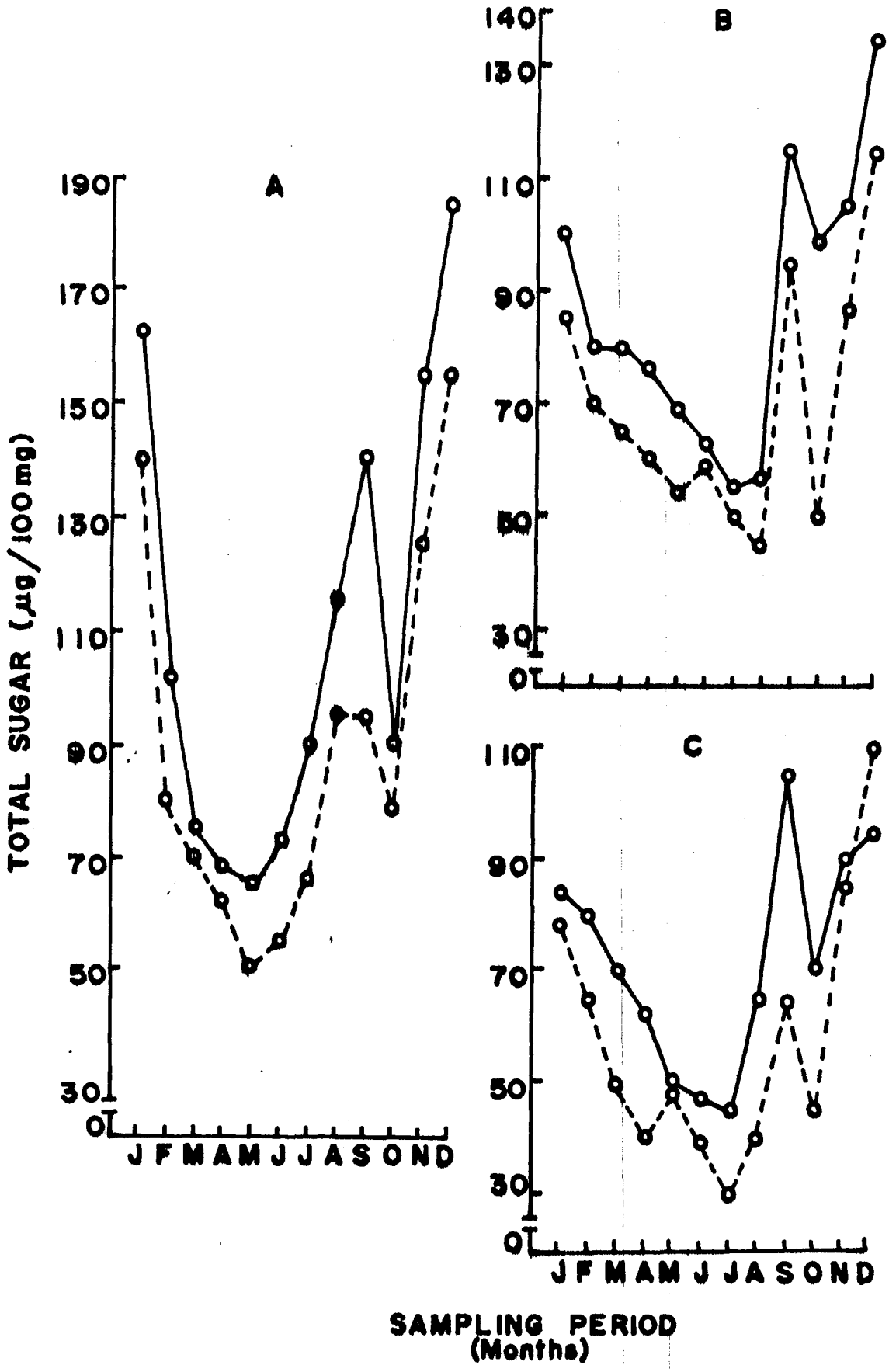

A
B
C
TOTAL SUGAR (µg/100mg)
190
170
150
130
110
90
70
50
30
0
140
130
110
90
70
50
30
0
110
90
70
50
30
0
J F M A M J J A S O N D
J F M A M J J A S O N D
SAMPLING PERIOD
(Months)

FIG. 3·4

Fig. 3.5 Seasonal variation of dehydrogenase activity in soil.

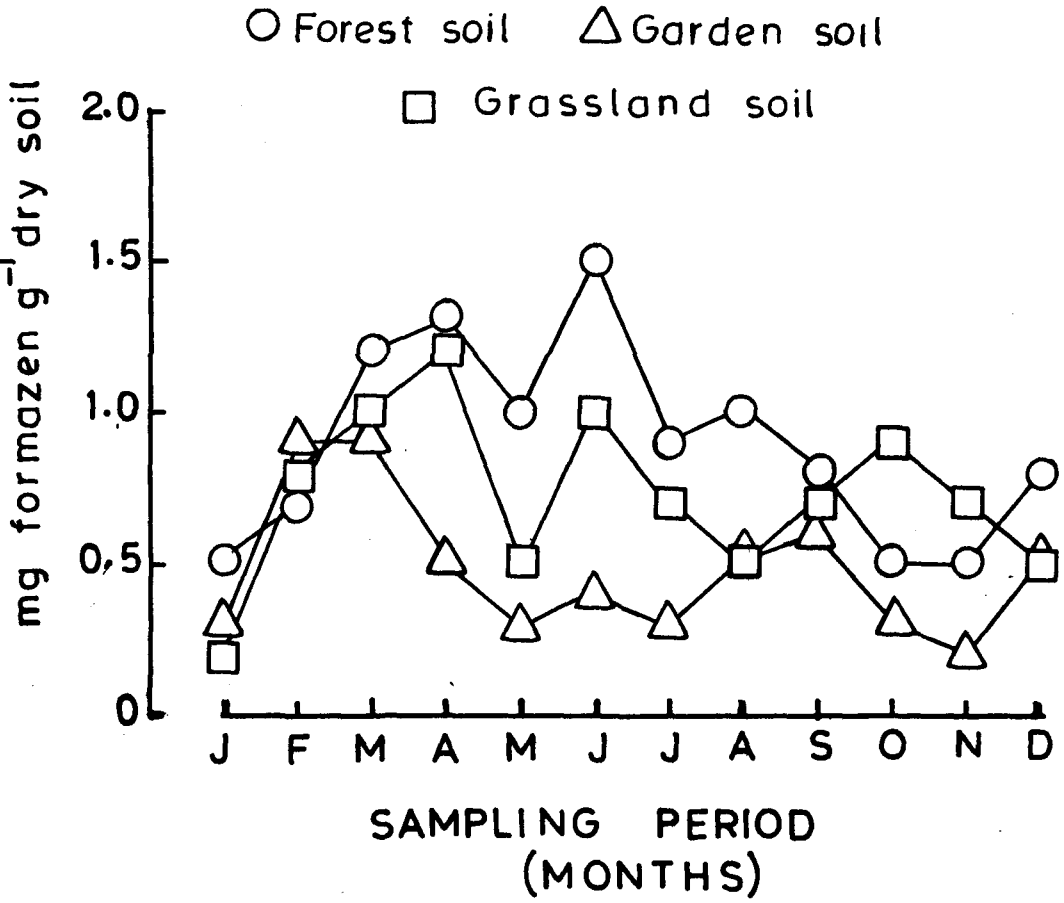

O Forest soil
△ Garden soil
□ Grassland soil
mg formazen g^{-1} dry soil
2.0
1.5
1.0
0,5
0
J F M A M J J A S O N D
SAMPLING PERIOD
(MONTHS)

FIG.3·5

tion in germination increased with the increase in concentration of ethrel amendments. Nutrient in different concentrations was found to counteract the inhibition (Plate 2). It was found that the glucose (1% and 10%) amendments nullified the inhibitory effect of ethrel in amended soil considerably. In case of glucose (10%) mixed with ethrel (100 µl/L), the percentage germination of sclerotium in forest, garden and grassland soil was recorded as 50%, 71.4% and 53.3% respectively. (Fig.3.6A,B,C)

(b) Sensitivity of conidia of **F. solani** to ethylene:

Ethrel in aqueous solution added to sterilised soil also inhibited the conidial germination and germ tube growth of **F. solani.** The inhibition increased with the increase in concentration. Highest inhibition in conidial germination and germ tube growth was 58.08% and 94.13% respectively (Table 1.4). Aqueous solution also show increased inhibition in germination with an increased concentration of ethrel (Table 1.4). Significant reduction in germ tube length was also recorded.

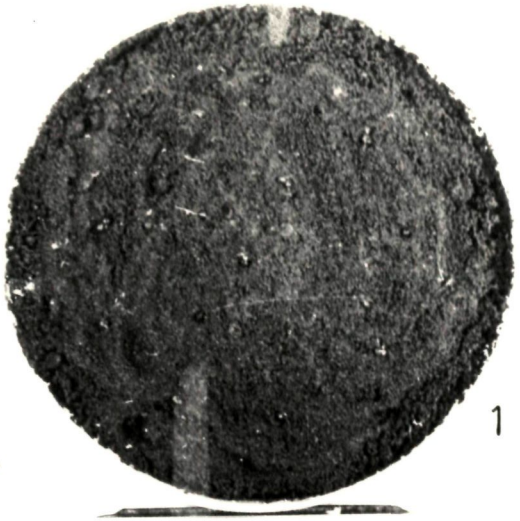
Ethrel induced inhibition and glucose (nutrient) stimulated conidial germination in aqueous solution and in sterilised soil when used separately. However, when they were mixed together in different proportions (i.e. 1% and 10% of glucose) the inhibition in conidial germination and germ tube length was found to be reduced significantly. The percentage

Plate 2: Showing induction of fungistasis in sterilised soil by ethrel (2 chloro ethane phosphonic acid) and its annulement by nutrient (i.e. glucose).

1) Natural (nonsterile) soil showing inhibition in sclerotial germination.
2) Sterilised soil showing maximum sclerotial germination.
3) Fungistasis reintroduced in sterilised soil by the amendment of ethrel.

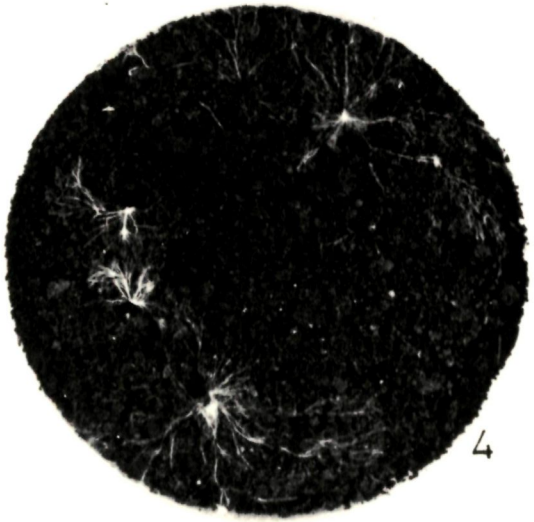
4 & 5) Annulement of ethrel induced fungistasis by supplementing glucose (1% and 10% respectively).

PLATE-2


1


2


3


4

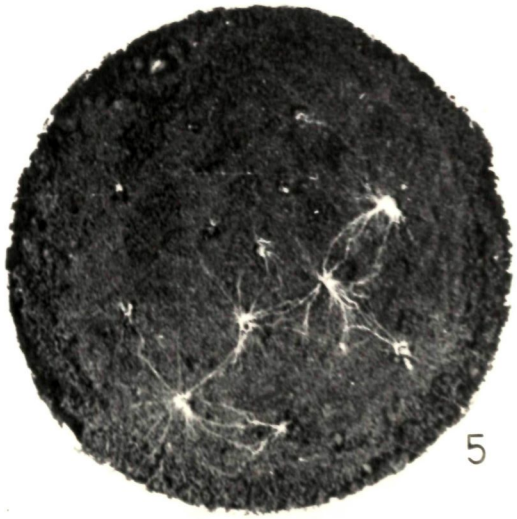

5

Fig.3.6 Effect of Ethrel (2 chloro ethane phosphonic acid) on the sclerotial germination of **Sclerotium rolfsii** in three different soil types were A = Forest, B = Garden and C = Grassland soil.

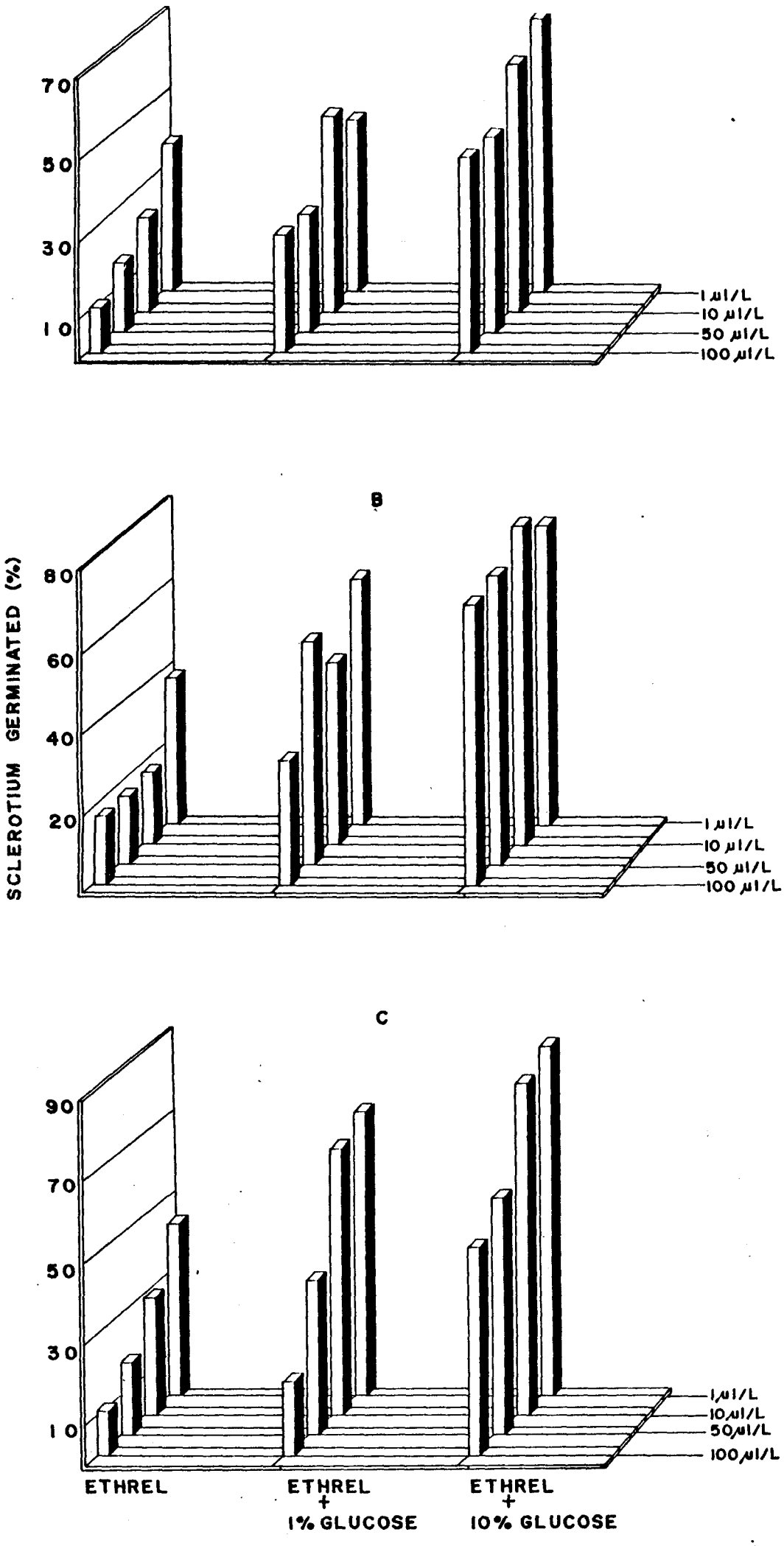

70
50
30
10
1 µl/L
10 µl/L
50 µl/L
100 µl/L
B
SCLEROTIUM GERMINATED (%)
80
60
40
20
1 µl/L
10 µl/L
50 µl/L
100 µl/L
C
90
70
50
30
10
1 µl/L
10 µl/L
50 µl/L
100 µl/L
ETHREL
ETHREL + 1% GLUCOSE
ETHREL + 10% GLUCOSE

FIG.3·6

TABLE 1.4 : Effect of Ethrel on spore germination and germ tube length of **Fusarium solani** in sterilised soil and aqueous solution.

Concentration (µl/L)	Ethrel effect		Inhibition	
	Spore germination (%) [1]	Germ Tube length (um) [2]	Spore Germination (%)	Germ Tube length (%)
Sterilised (Autoclaved) Soil				
Control	93.92	140.70±10.50	-	-
1	60.84	84.75±9.00	35.22	46.87
10	51.42	79.95±8.10	45.22	50.28
50	42.69	64.95±5.80	54.12	53.12
100	39.37	8.25±1.27	58.08	94.13
Aqueous Solution				
Control	83.51	272.24±1.49	-	-
1	72.50	121.18±1.03	13.18	55.50
10	50.00	71.38±0.71	40.12	73.80
50	16.30	66.40±0.89	80.48	75.60
100	4.00	49.80±0.52	95.21	81.70

1) Mean of 500 spores.
2) Mean of 25 germ tubes with ±S.E.

inhibition in conidia germination was recorded to be 57.2% and 48.4% in amended soil and 79.3% and 67.7% in aqueous solution respectively (Fig. 3.7 a,b) in case of ethrel (100µl/L) mixed with glucose (1 and 10%). Similar results were also observed in case of germ tube growth, where an increase in length was observed compared to control (i.e. ethrel only).

vii) **Effect on soil microbes**

It has been observed that the microbial population decreased significantly with an increase in concentration of ethrel (Table 1.5). **Penicillium** spp. **Aspergillus** spp. and **Fusarium** sp. are the dominant genera of fungi isolated from the amended soil.

viii) **Soil fungistasis in relation to organic and inorganic soil amendments**

All the organic and inorganic soil amendments in the present experiment, increased sclerotial germination, although, the degree of reduction in inhibitory effect varied.

The results indicate (Table 1.6) that the soil amendments partially reduced the soil fungistatic effect or in other words, partially nullified the inhibitory factor present in the soil, resulting increased germination of the sclerotium, compared tononamended control. A slight reduction in fungistatic effect was observed with **Eupatorium riparium** and **Helianthus annuus**, while **E. adenophorum** increased the fungistatic effect

Fig. 3.7 Effect of Ethrel and Ethrel + glucose in aqueous solution amended in sterilised soil (a) and in aqueous solution (b) on the spore germination and germ tube growth of **Fusarium solani.**

[Inhibition (%) in spore germination: ■——■ , Ethrel; □——□ , Ethrel + 1% glucose; Δ——Δ , Ethrel + 10% glucose. Inhibition (%) in germ tube growth: ■ - - ■ , Ethrel; □ - - □ , Ethrel + 1% glucose Δ - - Δ , Ethrel + 10% glucose.]

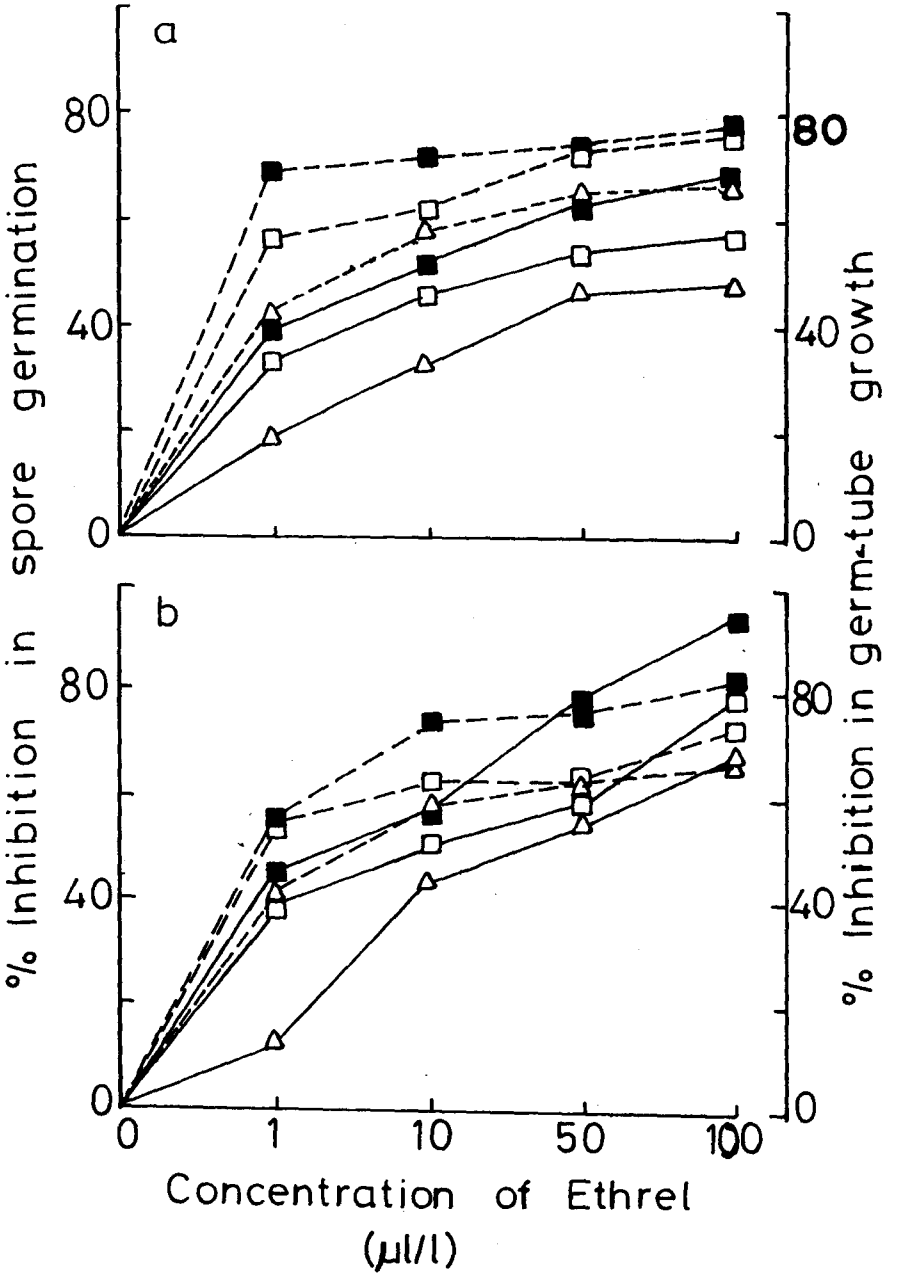

a
b
80
40
0
80
40
0
% Inhibition in spore germination
% Inhibition in germ-tube growth
0
1
10
50
100
Concentration of Ethrel
(µl/l)

FIG. 3·7

TABLE 1.5 : Effect of Ethrel (2 chloroethane phosphonic acid) on soil microbe population.[1]

Concentration (µl/L)	Fungi (10^4)	Actinomycete ($2x10^4$)	Bacteria ($3x10^4$)
Control	42	160	9 1
1	39	130	119
10	30	110	75
50	21	124	60
100	18	116	28
Significance (0.1%)	S	NS	S

1) Number of colonies g^{-1} dry soil.
S = Significant; NS = Not significant.

TABLE 1.6 : Effect of soil amendments on soil fungistasis.

Amendments	Concentration (%)	Inhibition in sclerotial germination(%)
ORGANIC		
Eupatorium adenophorum	1.00	65.00
	2.00	73.30
	3.00	78.30
Eupatorium riparium	1.00	56.70
	2.00	46.70
	3.00	66.70
Helianthus annuus	1.00	73.30
	2.00	73.30
	3.00	65.00
Pinus kesiya	1.00	58.30
	2.00	66.70
	3.00	66.70
Poultry litter	1.00	46.70
	2.00	61.70
	3.00	66.70
Control (without amendment)	-	100.00
INORGANIC		
Calcium carbonate	0.10	73.30
	0.25	73.30
	0.50	86.70
Calcium nitrate	0.10	80.00
	0.25	85.00
	0.50	86.70
Rock phosphate	0.10	80.00
	0.25	86.70
	0.50	86.70
Zinc sulphate	0.10	73.30
	0.25	85.00
	0.50	93.30
Urea	0.10	80.00
	0.25	86.70
	0.50	93.30
Ammonium nitrate	0.10	73.30
	0.25	86.70
	0.50	86.70
Control(without amendments)	-	100.00

1) Calculation based on 60 sclerotia in each case.

with the increase in the concentration of the leaf materials used as green manure. **Pinus kesiya** needles and poultry litter also partially reduced the fungistatic effect (33.3% to 53.3% germination) but in both the cases higher concentration promoted inhibition.

Most of the inorganic amendments used in this experiment, did not induce marked increase in sclerotial germination except in lower concentration (0.1% w/w), where 20 to 26.6% sclerotial germination was recorded compared to unamended control.

DISCUSSION

Fungistasis is a natural and seasonally variable inhibitory factor present in all types of soil, which supports microbial growth (Dobbs and Hinson, 1953, Jackson, 1957, Dobbs and Bywater, 1959; Dutta and Isaac, 1979). Higher fungistatic effect was observed to be imposed on the sclerotium during summer months (i.e. May-August). This inhibitory factor decreased with the decrease in atmospheric/soil temperature (i.e. in winter). No correlation between sclerotium germination and total microbial population was observed in case of forest soil, but a negative correlation between sclerotium germination and fungal ($r = -0.51$, $p = 0.1$) and actinomycete. ($r = -0.71$; $p = 0.01$) population was observed in case of grassland and garden soil, respectively.

All the three soil types tested in the present work showed higher population of **Penicillium** spp. throughout the year. This genus isknown to produce volatile and non-volatile antibiotics and might play a dominant role in soil fungistasis. Other soil fungi viz. **Aspergillus** spp., mucorales and **Fusarium** spp. were also found to be dominant in all the soil samples. **Aspergillus flavus** and **A. niger** were reported to induce fungistasis in soil (Mishra and Pandey, 1978) which supports the above view.

Moisture level of the soil was found to have direct influence on the degree of fungistatic activity in the three soil types, whereas, seasonal variation of organic matter and pH have no apparent effect. Lingappa and Lockwood (1964) also concluded that pH has no major role in soil fungistasis. It was also observed that the temperature to some extent has an effect on soil fungistasis, while rainfall and humidity apparently do not have any direct influence.

It has been reported by several workers that soil fungistatic/mycostatic effect is due to the loss of endogenous nurients from fungal propagules essential for germination which is utilised by the competing soil microorganisms (Ko and Lockwood, 1967; Yoder and Lockwood, 1973; Lockwood, 1977). This concept of rapid utilisation of energy yielding substrates including exudates from fungal propagules by soil microflora has been termed by Lockwood (1977) as 'microbial nutrient

sink'. On the other hand, nutrient independent propagules (capable of germinating without exogenous nutrient) germinate slightly in natural soil and also showed little germination when incubated on a sand bed undergoing continual leaching with water or salt solution (Ko and Lockwood, 1967; Hsu and Lockwood, 1973). This suppression in germination is due to nutrient depletion stress in continual leaching system, was related to loss of exudates from the spores (Bristow and Lockwood, 1975; Sneh and Lockwood, 1976; Filonow and Lockwood, 1979), which then require high energy yielding nutrients to annule mycostasis. The present result also suggests that soil fungistasis is nutrient dependent (have direct relationship with the sugar concentration in soil) as higher sclerotial germination (86.6% to 100%) in sterilised soil, is probably due to the release of sugars and other nutrients from the cells of dead soil microbes or may be due to the death of fungistatic metabolite releasing organisms.

Nutrient may release from sclerotia incubated in non sterile soil which might increase the activities of soil microorganisms resulting inhibition in sclerotial germination. Sclerotia of **S. rolfsii** has been observed to germinate independently without any external source of nutrient (Punja **et al**, 1984), but their prolonged exposure to non sterile soil, simultaneously with the loss of endogenous reserves through leakage, makes them to be nutrient dependent for germination

as observed for some other sclerotia forming fungi (Filonow and Lockwood, 1983) also. Rainfall induces loss of nutrient from the upper layer of soil might have helped in the loss of endogenous reserves from the propagules resulting inhibition in germination. The nutrients in terms of total sugar has been found to be seasonally variable and higher in sterilised soil compared to natural, which might be contributing to the seasonal variation of fungistasis in natural (unsterilised) soil. Winter months showed higher accumulation of sugar (155µg/100mg) in forest soil (natural) which was found to have direct relationship ($r = 0.624$; $p = 0.05$) with the sclerotial germination in soil. It has also been observed that a direct correlation exists between the sclerotium germination and the amount of sugar present in all sterilised soil tested (Table 1.7 to 1.9). The unavailable nutrients can be made available after sterilisation which might be utilised by the sclerotium of **S. rolfsii,** as a result the given soil shows lesser or no fungistatic effect.

Enzyme (dehydrogenase) activity in soil also show significant correlation with the sclerotial germination in soil. It has been observed to be directly correlated with the inhibition in sclerotial germination, in case of forest soil (Table 1.7) but no relationship in case of grassland (Table 1.9). It appears that the nutrient level (in terms of sugar) and moisture play a dominant role towards enzymatic activity and is significantly correlated in forest (Table

TABLE 1.7 : Test of significance between the different variables assessed in forest soil in relation to soil fungistasis.

Variables	Sclerotium germination (%)	Total microbial population g^{-1} dry soil			Total sugar in soil (µg/100mg)		Enzyme dehydrogenase (mg formazen g^{-1} dry soil)	Organic matter (%)	Soil pH	Soil moisture (%)
		Fungi (10^4)	Actino-mycete (10^4)	Bacteria (10^4)	Steri-lised	Non-ste-rilised				
	I	II	III	IV	V	VI	VII	VIII	IX	X
I	-	NS	NS	NS	S* (0.56)	S** (0.624)	S** (-0.58)	NS	NS	S*** (-0.85)
II	-	-	S* (0.57)	NS	NS	NS	NS	S** (0.66)	NS	NS
III	-	-	-	S** (0.64)	NS	NS	S* (-0.56)	NS	NS	NS
IV	-	-	-	-	NS	NS	NS	NS	NS	NS
V	-	-	-	-	-	S*** (0.97)	S** (-0.60)	NS	NS	S* (-0.52)
VI	-	-	-	-	-	-	S** (-0.59)	S*** (0.76)	NS	S** (-0.58)
VII	-	-	-	-	-	-	-	S** (-0.61)	NS	S** (-0.58)
VIII	-	-	-	-	-	-	-	-	NS	NS
IX	-	-	-	-	-	-	-	-	-	NS
X	-	-	-	-	-	-	-	-	-	-

Data showing astricks(*) marks are statistically significant (student T test) at different levels.
Significant at P=0.1(*); P=0.05(**); P=0.01(***) respectively.
Figure in the parenthesis indicates correlation co-efficient (r) value.
NS=Not significant.

TABLE 1.8 : Test of significance between the different variables assessed in garden soil in relation to soil fungistasis.

Variables	Sclerotium germination (%)	Total microbial population g^{-1} dry soil			Total sugar in soil (µg/100mg)		Enzyme dehydrogenase (mg formazen g^{-1} dry soil)	Organic matter (%)	Soil pH	Soil moisture (%)
		Fungi (10^4)	Actino-mycete (10^4)	Bacteria (10^4)	Steri-lised	Non-ste-rilised				
	I	II	III	IV	V	VI	VII	VIII	IX	X
I	-	NS	S*** (-0.71)	NS	NS	S* (0.52)	S* (0.57)	NS	NS	NS
II	-	-	NS	NS	NS	NS	S*** (-0.73)	NS	NS	NS
III	-	-	-	S** (0.69)	S*** (0.93)	NS	NS	NS	S*** (0.83)	NS
IV	-	-	-	-	NS	NS	NS	S** (0.62)	S** (0.63)	S* (0.56)
V	-	-	-	-	-	S*** (0.81)	NS	S** (0.61)	NS	NS
VI	-	-	-	-	-	-	NS	NS	NS	NS
VII	-	-	-	-	-	-	-	NS	NS	NS
VIII	-	-	-	-	-	-	-	-	NS	NS
IX	-	-	-	-	-	-	-	-	-	NS
X	-	-	-	-	-	-	-	-	-	-

Data showing astricks(*) marks are statistically significant (student T test) at different levels.
Significant at P=0.1(*); P=0.05(**); P=0.01(***) respectively.
Figure in the parenthesis indicates correlation co-efficient (r) value.
NS=Not significant.

TABLE 1.9 : Test of significance between the different variables assessed in grassland soil in relation to soil fungistasis.

Variables	Sclerotium germination (%)	Total microbial population g^{-1} dry soil			Total sugar in soil (µg/100mg)		Enzyme dehydrogenase (mg formazen g^{-1} dry soil)	Organic matter (%)	Soil pH	Soil moisture (%)
		Fungi (10^4)	Actinomycete (10^4)	Bacteria (10^4)	Sterilised	Non-sterilised				
	I	II	III	IV	V	VI	VII	VIII	IX	X
I	-	S* (-0.51)	NS	NS	S** (0.68)	S* (0.56)	NS	NS	NS	S*** (-0.73)
II	-	-	NS	NS	NS	NS	NS	NS	S** (-0.696)	NS
III	-	-	-	S*** (0.91)	NS	NS	NS	S** (0.62)	S** (0.66)	S** (-0.64)
IV	-	-	-	-	NS	NS	NS	S*** (0.73)	NS	NS
V	-	-	-	-	-	S*** (0.87)	NS	NS	NS	NS
VI	-	-	-	-	-	-	NS	NS	NS	NS
VII	-	-	-	-	-	-	-	NS	NS	S** (0.65)
VIII	-	-	-	-	-	-	-	-	NS	NS
IX	-	-	-	-	-	-	-	-	-	NS
X	-	-	-	-	-	-	-	-	-	-

Data showing astricks(*) marks are statistically significant (student T test) at different levels.
Significant at P=0.1(*); P=0.05(**); P=0.01(***) respectively.
Figure in the parenthesis indicates correlation co-efficient (r) value.
NS=Not significant.

1.7) and grassland soil (Table 1.9). Ross and Robert (1970) also found a significant correlation between dehydrogenase activity and soil moisture which supports the above findings.

Ramirez-Martinez and Mc. Laren (1966) and Dalal (1975) showed that moisture had a positive role in the growth, development and activity of soil microorganisms. In the present investigation, a positive correlation has been obtained between soil moisture and bacterial population in garden soil ($r = 0.56$; $p = 0.1$) (Table 1.8). Tate and Terry (1980) also reported similar results and concluded that moisture generally limits the microbial activity.

Ethrel (2-chloro ethane phosphonic acid) an ethylene generator (Warner and Leopold, 1969) in aqueous solution was found to induce fungistasis in sterilised soil which was otherwise non-fungistatic. It inhibits spore/sclerotia germination in soil, in aqueous solution even at lower concentration (1 µl/L). The inhibition increases with the increase in concentration of ethrel was observed with both the test organisms i.e. **Sclerotium rolfsii** and **Fusarium solani** in soil. The inhibition in spore germination was reduced by supplementing glucose (1% and 10%) in the experimental soil.

As observed in the present work, ethrel affect the microbial population in soil adversely. **Penicillium** spp. **Aspergillus** spp. and **Fusarium** sp. are the dominant genera isolated from the ethrel amended soil. The above findings

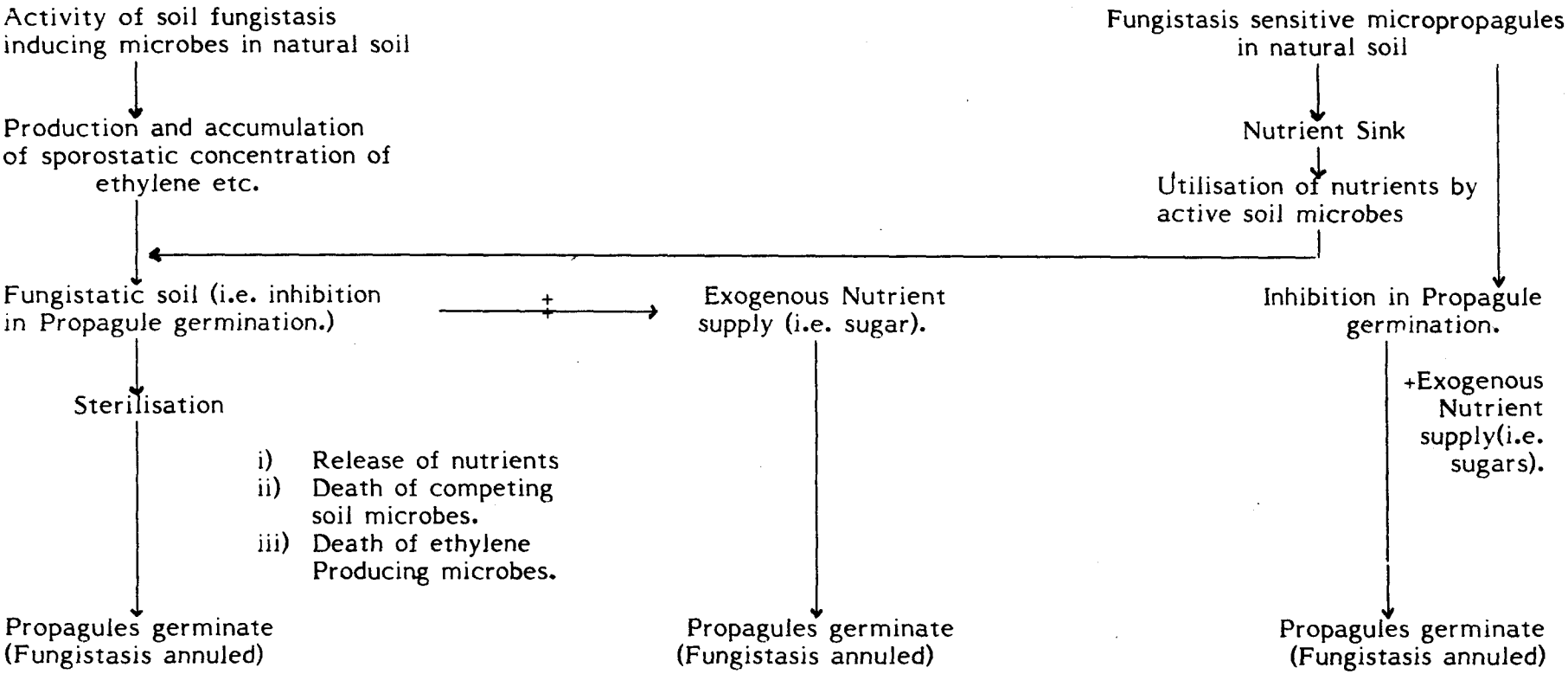
support the view of Smith (1973), that ethylene definitely affects the microbial population in soil and is a unique factor for determining whether or not a given soil is fungistatic. It has been observed earlier that there is a correlation between increased production of ethylene and greater microbial activity in top soil in relation to the increased fungistatic effect (Dutta **et al**, 1982). Some workers also reported that many soil microorganisms, mostly fungi produce ethylene in pure culture viz. **Penicillium digitatum** (Young **et al**, 1951), some **Penicillium** spp. (Considine and Patching, 1975); **Aspergillus clavatus** (Ilag and curtis, 1968); **Fusarium Oxysporum** and other **Fusarium** spp. (Swart and Kamerbeck, 1976), **Mucor hiemalis** and some yeast (Lynch, 1972). Several facultative anaerobic bacteria such as **Pseudomonas** spp. also have been found to produce ethylene at a high rate (Primrose and Dilworth, 1976; Primrose, 1976). The major amount of ethylene production in soil is possibly by those organisms sensitive to bacterial antibiotics and tolerant of 80°c moist heat for 30 minutes. This further strengthen the search for spore forming bacteria which significantly contributes to the ethylene production in soil and may have a definite role in soil fungistasis (Smith and Cook, 1974; Sutherland and Cook, 1980).

The ecological advantage of fungistasis with reference to soil-borne pathogens is mainly due to the imposed "exogenous dormancy" (Sussman, 1965). It is known that by adding organic

and inorganic amendments to the soil, the pathogenicity of fungi could be decreased, which again can be correlated with the increased population of actinomycetes and bacteria in the rhizosphere. Studies related to soil amendments with organic and inorganic substances, which helps in breaking the "exogenous dormancy" of fungal propagules in the soil may provide more information on the mechanism of plant disease control. Stimulation of sclerotial germination due to soil amendments (organic and inorganic) in the present study provides evidence on the involvement of nutrients in the reduction/annulement of soil fungistasis.

From the present observation and discussion, the probable pathway and the factors involved in fungistasis and its annulement in soil may be hypothetically proposed as follows.

THE PROBABLE PATHWAY OF SOIL FUNGISTASIS



PROBABLE FACTORS INVOLVED IN SOIL FUNGISTASIS AND ITS ANNULEMENT

FUNGISTATIC SOIL
(Factors involved)

Direct

i) Higher microbial activity

ii) Antagonistic microflora

iii)Hydrocarbons produced by microbes(i.e. ethylene)

iv) Anaerobic bacteria (prolific ethylene Producer)

Indirect

i) Soil temperature

ii) Soil moisture

iii) Available nutrients

iv) Organic matter in soil.

Fungistasis annuled/reduced by

i) Sterilisation

ii) Lower microbial activity

iii) Exogenous nutrient supply

iv) Organic soil amendments

v) Inorganic soil amendments.

Thus, it is evident from the present result that fungistasis in soil is biological in origin.

PART II PLANT AND SOIL RELATIONSHIP

CHAPTER II

EFFECT OF ORGANIC AMENDMENTS ON THE SOIL AND RHIZOSPHERE MICROFLORA IN RELATION TO THE BIOLOGY AND CONTROL OF SCLEROTIUM ROLFSII CAUSING FOOT ROT OF SOYBEAN.

INTRODUCTION

Several reports regarding organic amendments to soil in relation to disease development/control is available (Leach and Davey, 1942; Chowdhury, 1946a; Adams and Papavizas, 1969; Papavizas **et al**, 1968, 1970; Srivastava and Sinha, 1971). It is an established fact that the soil amendments affect the pathogen due to their direct toxic effect or by the production of toxic substances in the decomposition process (Leach and Davey, 1935; Henis and Chet, 1968; Chaudhuri and Maiti, 1978) and also indirectly by stimulating the antagonistic soil/rhizosphere microflora (Linderman and Gilbert, 1973a,b; Dutta and Isaac, 1979a,b). Soil amendments may also affect the interaction between the soil borne pathogen and soil microorganisms in plant debris and in the rhizosphere, causing a change in disease or in the survival ability of the pathogen (Garret, 1970).

Many crop residues are known to stimulate the activity of **Sclerotium rolfsii** in soil, possibly due to its high competitive saprophytic ability (Cooper, 1961; David and Rao, 1965; Sengupta and Roy, 1971). Some of the amendments such as compost (Mathur and Sinha, 1970), corn straw or oat (Mehrotra and Caludius, 1972; Gautum and Kolte, 1979) to soil decreased the disease incidence, through the release of ammonia (which is toxic to the pathogen) or by stimulating antagonistic microorganisms.

Higher plants are known to contain antifungal substances which have been found to be non-phytotoxic, systematic in nature, easily decomposed and stimulatory to host metabolism (Fawcett and Spencer, 1970; Beye, 1978; Tripathi **et al**, 1980). In contrast, many systematic fungicides impose harmful effect on the plant being phytotoxic (Fawcett and Spencer, 1970), due to long persistency (Beye, 1978) and cause pollutive effect (Dubey and Mall, 1972). Plant systems are also known to release chemicals, which are inhibitory to neighbouring plant species in soil (Rovira, 1969; Whittaker and Feeny, 1971). Most of the phytotoxic chemicals identified from the soil and from the decomposing crop residues, include short chain aliphatic fatty acids (Stevenson, 1967). Acetic, propionic and butyric acid are the primary phytotoxins released from the fermented suspension of wheat straw (Tang and Waiss, 1978).

It is a well known fact that the organic manures improve the physical properties of the soil, contributes nutrients and offer substrate for the microbes (Agarwal, 1967). During the decomposition process, organic amendment reduces the loss of nitrogen, caused by leaching (Pandey, 1975), thus acting as slow release fertilizer.

Reports are available on the plants belonging to Asteraceae (compositae) which produce substances (allelochemics) toxic to the germination and growth of other plants.

Some of the examples are **Helianthus annuus** (Wilson and Rice, 1968); **Chrysanthemum morifolium** (Kozel and Tukey, 1968); **Parthenium hysterophorus** (Sarma **et al**, 1976; Kanchan and Jaya Chandra, 1979); **Artemisia herba-alba** (Friedman **et al**, 1977); **Eupatorium riparium** (Rai and Tripathi, 1984); **Eupatorium adenophorum** (Tripathi **et al**, 1981).

Coniferous needles are also known to produce inhibitory substances mainly phenolics providing resistance to fungal colonization and growth (Barlocher and Oertli, 1978).

It was reported by Dutta and Isaac (1979a, b), Dutta (1981) that by influencing antagonistic genera of fungi in the host rhizosphere with organic and inorganic soil amendments, suppressed the severity of wilt disease of antirrhinum caused by **Verticillium dahliae.** In the present investigation, an attempt was made to observe the effect of organic amendments (viz. green leaves of **Eupatorium adenophorum, E. riparium, Pinus kesiya** needles, **Helianthus annuus** and poultry litter) on the soil and rhizosphere microflora in relation to the disease development/control of soybean (**Glycine max** L.) caused by **S. rolfsii** Sacc.

MATERIALS AND METHODS

Soybean and **S. rolfsii** as host and pathogen respectively were used in the present set of experiments.

Amendments used

The materials used in this work were easily available in and around Shillong (Meghalaya). These were fresh green leaves of the following plants,

1) **Eupatorium adenophorum** Spreng.

2) **Eupatorium riparium** Regel.

3) **Pinus kesiya** Royle ex Gordon.,

4) **Helianthus annuus** Linn.

Dried powdered poultry litter has also been used and following experiments were conducted **in vitro** and **in vivo.**

Effect of organic amendments on the growth of S. rolfsii in vitro

Fresh green leaves (25 gm approx.) of above mentioned plants were collected, surface sterilised with 0.1% mercuric chloride and after thorough washing, was crushed in a mortar and pastle. The leaf extract was then collected by passing through a clean muslene cloth and was considered as 100% concentration. Subsequently, different concentrations (1,2,3% v/v) of the extracts were prepared by adding appropriate amounts of the leaf extracts to the sterilised potato dextrose agar (PDA) medium (100 ml in each case) kept in asceptic

condition. 10ml of the medium having the leaf extracts were poured separately in the petridishes 9cm diameter) and was allowed to solidify. Dried and powdered poultry litter (1,2,3% w/v)was mixed with the sterilised medium similarly as mentioned above. A control set (i.e. without leaf extract/poultry litter) was also maintained. Replicates were made for all treatments and control. A 4mm disc of the test pathogen (**S. rolfsii**) was inoculated and radial expansion was recorded after required period of incubation as described before (page 34).

Effect of leaf/litter extracts on sclerotium germination

To observe the effect of leaf extracts (in aqueous solution) on the sclerotial germination, the extracts prepared as mentioned above were mixed separately with sterile distilled water to get the required concentration to be used (i.e. 1,2,3% v/v). The aqueous extract of the poultry litter was prepared by boiling diferent concentration (1,2,3% w/v) of the litter in distilled water (100 ml). To these aqueous solutions, sclerotium of **S. rolfsii** were added and kept for 7 days at environmental temperature. Petri plates containing PDA medium and plant extract/poultry litter were also prepared as mentioned before were inoculated with sclerotia. Percentage of sclerotial germination was recorded following the method of Agnihotri **et al** (1975) (as described in page 41) after 7 days of incubation in aqueous solution and also by direct observation.

Effect of organic amendments on the survibality of sclerotium in soil

A thorough mixing of the soil and the chopped (0.5cm approx.) green leaves of the plants (i.e. **Eupatorium adenophorum, E. riparium, Helianthus annuus, Pinus kesiya** needles) and powdered poultry litter in different concentrations were used separately for the respective experiments. The amended soil kept in pots (plastic glasses) were maintained at 50% moisture level and was left for 15 days for decomposition. Thirty mature sclerotia was then mixed thoroughly with soil and was left for incubation. Percentage of the survived sclerotia was calculated after the predetermined period of incubation (i.e. 15,30,45,60 days respectively).

Effect on soybean seed germination

Effect of organic amendments (i.e. leaf extracts and poultry litter) on soybean seed germination and radicle growth was determined following the method as described earlier (page 35).

Toxicity of the leaf/poultry litter extracts to soybean seedlings

Different concentrations (v/v) of leaf extracts were made with Knop's normal solution. Powdered poultry litter was added directly to the Knop's normal solution in different proportions (1,2,3% w/v). Phytotoxicity was recorded after 48 hours of root dip treatment of the one month old soybean seedlings.

Estimation of total sugars, phenols and amino acids in the treated soybean radicles in vitro

Soybean seedlings were grown in the moist chamber treated with different concentrations of the leaf / poultry litter extracts separately and when they were one week old, total sugars, amino acids and phenols were estimated from the radicles following the method described by Peach and Tracey (1955) and Bray and Thrope (1954) respectively. (Page 43-45).

Effect of amendments on the soil microorganisms

The effect of amendments on the test pathogen (i.e. **S. rolfsii**) and other microorganisms in the **S. rolfsii** infested soil was carried out at ICAR farm, Barapani, Shillong.

The chopped (0.5 cm approx. size) green leaves of different plant materials (as described earlier) were mixed thoroughly at different concentrations (i.e. 1,2,3% w/w) separately, with the infested soil kept in pots (22cm. diameter). Powdered poultry litter was added directly to the soil. The amended soil in pots were then left for 15 days at 50% moisture level for decomposition. An unamended infected control was also maintained. Total microbial population together with percentage of relative abundance of particular genera/species of fungi was calculated following a modified soil dilution plate method as described earlier (page 37).

Effect of soil amendments on the rhizosphere microflora

Soybean seeds were sown in the soil after 15 days of soil amendments. Non germinated seeds were detected and was taken out from the soil and was kept in a moist chamber for further laboratory observation.

Isolation of rhizosphere microflora of one month old seedlings were done following the modified method of Timonin (1940) as described by Baruah and Dutta (1978). The dilutions used for fungi, actinomycete and bacteria were, 1/10,000; 1/20,000 and 1/30,000 respectively. The 'rhizosphere effect' (R.E.) was also calculated. From the total fungal population, percentage of relative abundance of a specific fungus was calculated.

Assessment on disease severity

From a preliminary observation it was noted that the first symptom in soybean seedlings appeared, when they were three weeks old. The infection appeared first in the cotyledon followed by light wilting symptom of seedlings, which subsequently revives, followed by the fall of the cotyledon. Visual record of the seedling infection was made from the fifth week onwards on a weekly basis. The percentage of disease severity/disease control was calculated on the basis of the number of plants showing symptoms compared to the total number of plants grown in each treatment.

Population dynamics of Sclerotium rolfsii in amended soil

Monthly variation in the population of **Sclerotium rolfsii** in the soil amended with organic materials was determined for a complete cropping season using slightly modified selective medium as proposed by Backman and Rodriguez-Kabana (1976) for **S. rolfsii**. (page 42).

Effect of amendments on plant height

Seedling /plant height of soybean was recorded on a weekly basis till the plant achieved its maximum height.

Plants dry matter accumulation and yield assessment

Soybean plants were harvested at the time when the pods were matured and ripe. Total dry matter accumulation (i.e. dry weight of root, shoot and pod /plant) was calculated from the harvested plant.

Yield/plant was also calculated by taking the mean weight of seeds (gm) from the plants of each treatment/ control.

Application of some promising organic amendments in the experimental field

A farm site having **S. rolfsii** infested natural soil was selected for the experimentation. All the plots were made (5 sq.m) in a single terrace. Organic amendments i.e.

green leaves of **Eupatorium adenophorum** and **E. riparium** cut into 0.5cm size, was amended to soil at the rate of 10, 20, 40q/ha and was left for decomposition for two weeks. Randomised block design (RBD) was used for this experiment.

Eight rows were made in each plot (5 sq m) and seeds (variety 'bragg') were sown at a spacing of 5-7cm. Effect of green manures were assessed on the following parameters:

(a) Monthly variation in the population of **S. rolfsii** in the amended soil (method described in page 42).

(b) Seedlings/plants height (fortnightly).

(c) Disease severity (assessed fortnightly).

(d) Yield (q/ha).

RESULTS

Among the organic amendments used only **Eupatorium adenophorum** and **E. riparium** leaf extract inhibited the growth of **S. rolfsii in vitro**. Highest inhibition (52.2%) in radial growth was observed with **E. adenophorum** (3% v/v) followed by **E. riparium** (47.7% in 3% v/v) (Fig. 4.1). Other amendments showed very little inhibitory effect. Higher concentration of the plant extracts also showed inhibitory effect on the germination of sclerotia. The maximum reduction in germination was observed with pine neddle extract (3% v/v) (Table 2.1).

Fig.4.1 Effect of organic amendments (plant/litter extract):
A = **Eupatorium adenophorum,** B = **Eupatorium riparium,** C = **Helianthus annuus,** D = **Pinus kesiya,** E = Poultry litter, on the growth of **Sclerotium rolfsii in vitro.**

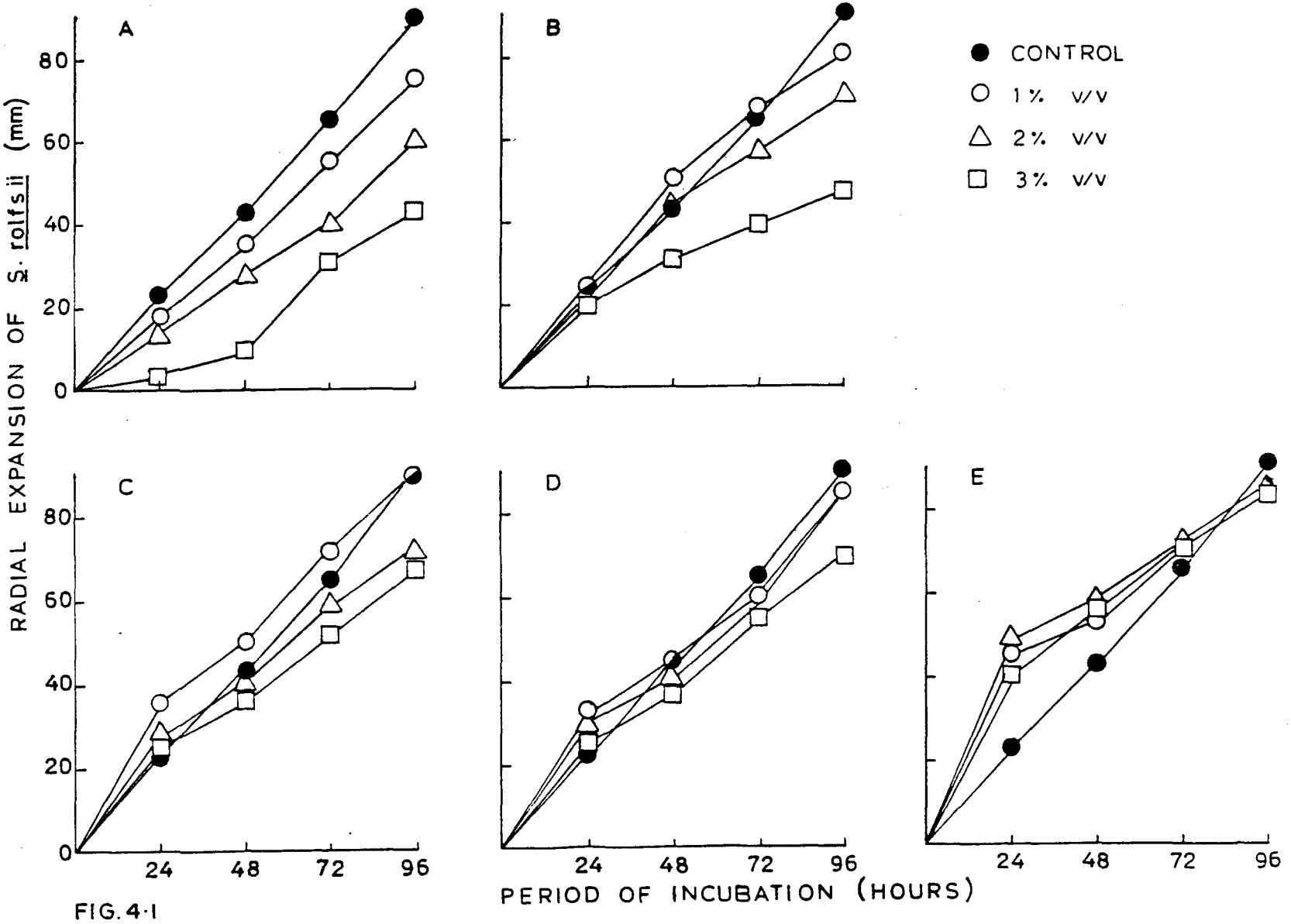

A
B
C
D
E
CONTROL
1% v/v
2% v/v
3% v/v
RADIAL EXPANSION OF S. rolfsii (mm)
0
20
40
60
80
24
48
72
96
PERIOD OF INCUBATION (HOURS)

FIG. 4·1

TABLE 2.1 : Effect of Organic amendments on the survibality of Sclerotium.

Amendments	Concentration (%)	Germination in PDA[1] amended with plant/litter extract(%)	Sclerotial germination(%)				
			In aqueous solution after 7 days[1]	In soil[2] (Period of Incubation(Days))			
				15	30	45	60
Eupatorium adenophorum	1	100.00	100.00	100.00	100.00	100.00	100.00
	2	100.00	100.00	100.00	100.00	100.00	100.00
	3	80.00	85.00	100.00	100.00	100.00	100.00
Eupatorium riparium	1	100.00	100.00	100.00	100.00	83.80	63.60
	2	100.00	100.00	100.00	100.00	81.30	68.40
	3	100.00	70.00	100.00	100.00	61.90	25.00
Helianthus annuus	1	100.00	100.00	100.00	75.00	57.10	20.00
	2	100.00	100.00	100.00	60.00	28.60	6.60
	3	100.00	80.00	80.00	60.00	27.20	-
Pinus kesiya	1	100.00	100.00	100.00	100.00	100.00	63.60
	2	100.00	100.00	100.00	90.90	80.00	50.00
	3	70.00	61.76	100.00	90.00	42.90	29.40
Poultry litter	1	100.00	93.33	100.00	100.00	100.00	81.80
	2	100.00	100.00	100.00	100.00	75.00	73.30
	3	100.00	83.30	66.60	50.00	27.20	-
Control (without amendment)	-	100.00	83.30	100.00	100.00	100.00	100.00

1) Calculation based on 60 sclerotia in each case.
2) Calculation based on recovered sclerotia.
(-) No germination.

Survibality

In the viability test, it was observed that the soil amendment with **E. adenophorum** did not affect the viability of sclerotium during the experimental period (upto two months), while other amendments (i.e. **E. riparium, Pinus kesiya, Helianthus annuus** and Poultry litter) decreased the sclerotial viability in soil with the increased amount of organic amendments and incubation period. Highest reduction in sclerotial germination was observed in case of **Helianthus annuus** and poultry litter after 45 days of incubation (27.2% germination) at higher concentration (3% w/w). Further increase of incubation period resulted complete loss in viability of sclerotium (Table 2.1). The recovery of empty sclerotial shell from the soil in most of the cases suggest , their germination during the incubation period in the soil, whereas in control (non amended) soil, most of the sclerotium remained viable upto two months of experimentation.

Effect on seed germination and radicle growth

Leaf extracts and poultry litter water extract did not show any adverse affect on the germination of soybean seeds **in vitro. Eupatorium adenophorum, Helianthus annuus** and higher concentration of **Pinus kesiya** needle extracts (3%v/v) were found to be slightly inhibitory, whereas, poultry litter was found to stimulate the radicle growth (Table 2.2).

TABLE 2.2 : Effect of plant extracts/poultry litter on the germination & radicle growth of soybean seeds.

Plant/poultry litter extracts	Concentration (%)	Effect of leaf/litter extracts **in vitro**			Effect of soil amendments **in vivo**	
		Seed germination (%)[1]	Radicle growth with ±S.E. (Cm)[1]	Phyto-toxicity[2]	Seed germination (%)[3]	Pre-emergence rot (%)[3]
Eupatorium adenophorum	1	100	8.42±1.30	-	81.60	18.40
	2	100	7.19±1.64	-	86.60	13.40
	3	100	7.69±1.69	-	83.30	16.70
Eupatorium riparium	1	100	11.75±1.60	-	90.00	10.00
	2	100	12.38±1.91	-	76.60	23.40
	3	100	9.83±2.85	-	81.60	18.40
Helianthus annuus	1	100	7.06±2.08	-	78.30	21.70
	2	100	10.00±2.11	-	90.00	10.00
	3	100	6.80±1.73	-	75.00	25.00
Pinus kesiya	1	100	12.75±0.96	-	80.00	20.00
	2	100	11.00±0.82	-	88.30	11.70
	3	100	7.14±1.00	+	88.30	11.70
Poultry litter	1	100	13.00±1.76	-	93.30	6.70
	2	100	9.86±1.17	-	85.00	15.00
	3	100	11.67±0.99	-	88.30	11.70
Control (without treatment)	-	100	9.65±2.25	-	86.66	13.40

1) Calculation based on 20 seeds.
2) Obserevation made from 10 exposed seedlings; (-) non toxic; (+) toxic.
3) Calculated from 60 seeds.

Phytotoxicity

Organic amendments did not show any toxic effect on the soybean seedlings, grown in Knop's normal solution amended with the extracts. **Pinus kesiya** needle extract (3% v/v) showed very little toxicity. Scorching of the leaves was also observed in this case (Table 2.2).

Estimation of total sugars, phenols and amino acids

Accumulation of sugars in most of the treated radicles decreased with the increase in concentration of the extract excepting **Pinus kesiya** and poultry litter treatment, where variation in sugar accumulation was recorded. Only **E. riparium** treatment increased amino acids, whereas a significant increase in phenolic compounds were recorded in the tissue of all the treated soybean radicles (Table 2.3).

Effect of organic amendments on the soil microorganisms

A qualitative and quantitative change in microbial population was observed due to organic amendments to soil. An increase in the microbial population (i.e. fungi, actinomycete and bacteria) in general was observed with a few exceptions. It was further observed that the higher concentration of the amendments (i.e. 3% w/w) were found to have inhibitory effect on the fungal population compared to the lower concentration (i.e. 1% w/w). Only **E. adenophorum** and **E. riparium** amend-

TABLE 2.3 : Estimation of total sugars, phenols and amino acids (µg/100 mg dry wt)[1] in soybean radicles grown in the aqueous solution with plant extract/poultry litter **in vitro.**

Plant extract/ poultry litter	Concentration(%)	Total sugars	Total phenols	Total amino acids
Eupatorium adenophorum	1	280.00	8.00	110.00
	2	185.00	10.00	140.00
	3	100.00	10.00	150.00
Eupatorium riparium	1	195.00	6.00	300.00
	2	140.00	7.00	340.00
	3	135.00	11.00	290.00
Helianthus annuus	1	190.00	4.00	180.00
	2	185.00	5.00	156.00
	3	160.00	5.00	140.00
Pinus kesiya	1	170.00	11.00	200.00
	2	207.50	9.00	200.00
	3	80.00	9.00	130.00
Poultry litter	1	120.00	7.00	140.00
	2	175.00	9.00	110.00
	3	130.00	10.00	110.00
Control (without treatment)	-	220.00	4.00	240.00

1) Mean of three replicates in each case.

ments to soil decreased the bacterial population singificantly (Table 2.4).

Effect on the particular genera of fungi in soil

It is evident from the Table 2.5 that a considerable increase in the Aspergilli population occurred in the soil due to **Helianthus annuus** and **Pinus kesiya** needle amendments. Other amendments i.e. **E. adenophorum** (3% w/w) and poultry litter (2% w/w) also increased Aspergilli population. Altogether seven **Aspergillus** spp. were isolated from the soil, out of which **A. flavus** and **A. niger** were found to be the dominant. Poultry litter (3% w/w) greatly increased mucorales, whereas **E. riparium** boosted **Penicillium** spp. and **Trichoderma** spp. especially **Penicillium rubrum** and **Trichoderma harzianum.** Thirty eight species belonging to eighteen genera of fungi were isolated from the amended soil (Table 2.6).

Effect on the rhizosphere

Among the organic amendments used, only **E. riparium** (1% w/w) amendment increased the fungal population, while, rest of the amendments reduced fungi and actinomycete (excepting **E.adenophorum**, 3% w/w only) in the soybean rhizosphere. In general a significant increase in bacterial population was observed due to organic amendments to soil (Table 2.7).

TABLE 2.4: Effect of Organic amendments on the soil micro-organisms.

Amendments	Concentration (%)	Total soil microflora in thousands g^{-1} dry soil[1]		
		Fungi (10^4)	Actinomycete (2×10^4)	Bacteria (3×10^4)
Eupatorium adenophorum	1	26.60	69.60	39.00
	2	13.75	81.00	40.67
	3	12.50	67.70	37.50
Eupatorium riparium	1	18.00	88.95	40.60
	2	24.60	65.00	52.50
	3	9.58	57.70	36.00
Helianthus annuus	1	18.30	113.50	140.70
	2	12.00	122.50	71.93
	3	8.75	179.95	63.60
Pinus kesiya	1	15.80	81.25	98.97
	2	13.75	151.00	91.30
	3	9.60	159.15	93.00
Poultry litter	1	11.70	133.50	77.70
	2	14.58	139.00	81.40
	3	15.00	167.50	86.10
Infected control (without amendment)	-	10.40	62.50	44.20
Significance by F test (5%)	-	Yes	Yes	Yes

1) Mean of three replicates in each case.

TABLE 2.5 : Effect of organic amendments on the percentage of relative abundance of different genera of fungi in Pathogen infested soil

Amendments (leaf/litter)	Concentration (%)	Aspergilli	Cephalosporium spp.	Cladosporium spp.	Fusarium spp.	Geotrichum spp.	Gliocladium spp.	Mucorales	Penicillia	Trichoderma spp.	Yeast	Other genera of fungi imperfecti
Eupatorium adenophorum	1	8.57	-	8.57	-	-	-	16.70	34.20	11.40	14.28	5.70
	2	-	11.10	-	7.40	11.10	7.40	7.40	25.90	7.40	18.51	3.70
	3	33.38	12.12	-	6.06	3.03	-	-	12.12	9.09	18.18	6.06
Eupatorium riparium	1	9.09	4.54	-	4.54	-	-	-	40.88	9.09	25.00	6.80
	2	11.20	5.60	8.33	-	-	-	15.60	37.33	22.20	8.33	2.80
	3	6.25	-	-	-	-	-	-	68.75	18.75	-	6.25
Helianthus annuus	1	32.34	-	-	14.70	8.82	-	5.88	11.76	5.88	14.70	5.88
	2	55.04	-	-	-	-	-	10.32	24.08	3.44	-	6.88
	3	42.84	4.76	4.76	4.76	4.76	-	9.52	9.52	4.76	4.76	9.52
Pinus kesiya	1	31.48	7.89	5.26	-	13.15	-	15.78	18.41	2.63	-	5.26
	2	18.18	2.30	6.06	6.06	-	6.06	15.15	12.12	12.12	15.15	6.06
	3	26.07	-	2.17	6.51	8.68	-	21.70	17.38	13.02	-	6.51
Poultry litter	1	14.28	7.14	3.57	7.14	10.71	3.57	7.14	10.71	7.14	17.85	10.71
	2	28.50	-	-	-	19.95	-	17.10	8.55	11.40	14.25	-
	3	13.90	-	-	13.90	-	-	22.23	27.83	13.90	-	8.33
Control (without amendments)	-	16.80	-	-	-	19.60	5.60	5.60	27.80	2.80	14.00	8.40

TABLE 2.6 : Effect of organic amendments to soil on the percentage of relative abundance of fungi under infested condition.

Soil Fungi	Control (without amend-ment)	Eupatorium adenophorum			Eupatorium riparium			Pinus kesiya			Helianthus annuus			Poultry litter		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Aspergillus candidus Link ex Fries.	-	-	-	12.12	-	-	-	5.26	3.03	8.68	-	10.32	19.04	7.14	-	-
A. flavipes Thom & Church.	8.40	-	-	-	-	-	-	5.26	12.12	-	11.76	-	-	-	-	-
A. flavus Link ex Fries.	-	8.57	-	6.06	9.09	5.60	6.25	15.70	-	13.02	14.70	6.88	9.52	-	8.55	13.90
A. niger Van. Tieghem.	8.40	-	-	15.20	-	2.80	-	5.26	3.03	4.34	-	17.20	14.28	7.14	11.40	-
A. repens (corda) da Bary.	-	-	-	-	-	-	-	-	-	-	5.88	-	-	-	-	-
A. versicolor (Vuillemin) Tiraboschi.	-	-	-	-	-	-	-	-	-	-	-	20.64	-	-	-	-
Aspergillus sp.	-	-	-	-	-	2.80	-	-	-	-	-	-	-	-	8.55	-
Cephalosporium roseogriseum Saksena.	-	-	11.10	12.12	4.54	5.60	-	7.89	2.30	-	-	-	4.76	7.14	-	-
Cladosporium herbarum Link ex. Fries.	-	8.57	-	-	-	8.33	-	5.26	6.06	2.17	-	-	4.76	3.57	-	-
Cunninghamella sp.	-	5.70	-	-	-	-	-	-	-	-	5.88	-	9.52	7.14	-	-
Fusarium oxysporum	-	-	7.40	7.40	4.54	-	-	-	6.06	6.50	14.70	-	4.76	7.14	-	13.90
Geotrichum sp.	19.60	-	11.10	3.03	-	-	-	13.15	-	8.68	8.82	-	4.76	10.71	19.95	-
Gliocladium catenulatum Gilman & Abbott.	5.60	-	7.40	-	-	-	-	-	6.06	-	-	-	-	3.57	-	-
Helminthosporium sp.	-	-	-	-	-	-	6.25	-	-	-	-	3.44	-	-	-	-
Humicola grisea Traaen.	-	5.70	-	-	-	5.60	-	-	-	8.68	-	-	-	7.14	-	-
Mucor cercinelloides Van Tieghem.	-	-	7.40	-	-	-	-	2.63	6.06	-	-	-	-	-	8.55	-
M. plumbeus Bonorden.	-	-	-	-	-	-	-	5.26	-	2.17	-	3.44	-	-	-	-
Mucor sp.	2.80	11.00	-	-	-	-	-	-	-		2.94				8.55	13.90
Penicillium brefeldianum Dodge.	5.60	14.20	18.50	-	9.09	-	-	10.52	6.06	8.68	-	6.88	4.76	-	-	-
P. chrysogenum. Thom.	-	5.70	-	-	6.80	-	12.50	7.89	-	8.68	-	-	4.76	7.14	-	13.90
P. digitatum. Saccardo.	-	-	-	-	-	-	12.50	-	-	-	-	-	-	-	-	5.60
P. granulatum Bainier.	-	-	3.70	-	-	5.60	-	-	-	-	-	-	-	3.57	-	-
P. implicatum Biourge.	-	-	-	-	-	-	6.25	-	-	-	-	-	-	-	-	8.33
P. oxalicum Thom.	-	-	-	-	9.09	-	6.25	-	-	-	-					
P. rubrum stoll.	11.10	14.30	-	9.09	-	23.40	18.75	-	6.06	-	-	10.32	-	-	8.55	-
P. vermiculatum Dangeard.	11.10	-	3.70	3.03	15.90	8.33	6.25	-	-	-	11.76	6.88	-	-	-	-
Penicillium sp.	-	-	-	-	-	-	6.25	-	-	-	-	-	-	-	-	-
Phoma sp.	-	-	3.70	-	-	-	-	-	-	-	-	-	-	3.57	-	-
Pythium sp.	8.40	-	-	-	6.80	-	-	5.26	-	-	-	3.44	-	-	-	-
Rhizopus stolonifer (Ehrenb ex Link)Lind. 1913	2.80	-	-	-	-	-	-	7.89	9.09	8.66	2.94	6.88	9.52	-	-	8.33
Thielaviopsis basicola (Berk & Br)Zopf.	-	-	-	6.06	-	2.80	-	-	3.03	-	-	-	-	-	-	-
Torula sp.	-	-	-	-	-	-	-	-	3.03	6.57	-	-	-	-	-	8.33
Trichoderma aureoviride Rifai.	-	-	-	-	-	-	-	-	12.12	-	-	-	-	-	-	-
T. harzianum. Rifai.	-	-	-	-	-	22.20	18.75	-	-	-	-	-	4.76	3.57	-	-
T. koningii oudemans.	2.80	5.70	-	-	-	-	-	-	-	8.68	-	-	-	3.57	8.55	-
T. viride Pers ex. Fries.	-	5.70	7.40	9.09	9.09	-	-	-	-	4.34	5.88	3.44	-	-	2.85	13.90
Trichoderma sp.	-	-	-	-	-	-	-	2.63	-	-	-	-	-	-	-	-
Yeast (red)	14.00	14.28	18.51	18.18	25.00	8.33	-	-	15.15	-	14.70	-	4.76	18.85	14.25	-

Amendments applied at a rate of 1, 2, 3% w/w.

TABLE 2.7 : Effect of Organic soil amendments on the rhizosphere microflora of soybean grown in pathogen infested soil.

Soil amendments	Concentration (%)	Rhizosphere micro-organisms in thousands g^{-1} dry soil[1]			Rhizosphere effect (RS/CS)[2]		
		Fungi (10^4)	Actinomycete ($2x10^4$)	Bacteria ($3x10^4$)	Fungi	Actinomycetes	Bacteria
Eupatorium adenophorum	1	14.38	383.00	466.70	0.72	1.14	2.04
	2	15.50	297.50	393.30	0.78	0.89	1.72
	3	21.60	443.00	449.60	1.08	1.32	1.97
Eupatorium riparium	1	46.80	244.00	251.60	2.34	0.73	1.10
	2	35.80	235.00	320.80	1.79	0.70	1.40
	3	37.60	337.50	367.50	1.88	1.00	1.61
Helianthus annuus	1	18.00	255.00	775.00	0.90	0.76	3.39
	2	20.00	358.00	903.00	1.00	1.37	3.95
	3	29.00	394.00	628.30	1.45	1.18	2.75
Pinus kesiya	1	24.00	89.50	685.67	1.20	0.27	2.99
	2	19.00	121.00	700.30	0.95	0.36	3.06
	3	11.00	200.00	735.00	0.55	0.60	3.20
Poultry litter	1	25.70	243.50	484.60	1.29	0.73	2.12
	2	16.00	165.50	501.70	0.80	0.49	2.19
	3	12.00	150.00	532.70	0.60	0.45	2.33
Control rhizosphere soil	-	36.59	400.00	270.80	1.82	1.19	1.18
Control soil	-	20.00	335.00	228.75	-	-	-
Significance by F test (1%)	-	Yes	Yes	Yes			

1) Mean of replicates in each case.
2) Rhizosphere soil/control soil.

Effect on the particular genera of rhizosphere fungi

E. adenophorum (2% w/w) increased **Penicillium** spp. in the rhizosphere, while, **Aspergillus** spp., mucorales and **Trichoderma** spp. were found to be stimulated by all treatments (Table 2.8). A comparatively higher population of **Aspergillus** spp. was observed with **E. riparium** (2% w/w) and **E. adenophorum** (3% w/w) amendments. Among the **Trichoderma** spp., **T. harzianum** and **T. koningii** were found to be the dominant species in the soybean rhizosphere following **Eupatorium riparium** soil amendments. Altogether thirty two species belonging to fourteen genera have been isolated from the rhizosphere (Table 2.9). Other genera i.e. **Cephalosporium, Cladosporium** and fungi imperfectii, also showed fluctuations in their population in the rhizosphere. Few unidentified imperfect genera fungi have also been isolated from the rhizosphere of soybean.

Assessment of disease severity

It was observed that the non germinated soybean seeds in the soil were infected with **S. rolfsii**. Superficial mycelial growth of **S. rolfsii** was observed invariably on the seeds, when kept in moist chamber. Sowing of seeds followed by the soil amendments might have been the cause of the seed rot, as rapid multiplication of the sclerotium population was observed following soil amendments. Highest seed rot was observed with **Eupatorium riparium** (2 & 3% w/w), **Helianthus annuus** (1 & 3%w/w) and **Pinus kesiya** needle (1% w/w) soil amendments (Table 2.2).

TABLE 2.8 : Effect of organic amendments on the percentage of relative abundance of different genera of fungi in soybean rhizosphere grown in infested soil.

Amendments (leaf/litter)	Concentration (%)	Aspergilli	Cephalosporium spp.	Cladosporium spp.	Fusarium spp.	Geotrichum spp.	Gliocladium spp.	Mucorales	Penicillia	Trichoderma spp.	Yeast	Other genera of fungi imperfecti
Eupatorium adenophorum	1	10.25	-	-	5.12	10.25	-	-	48.66	2.56	12.80	10.25
	2	8.10	8.10	-	13.50	-	-	5.40	64.83	-	-	-
	3	35.00	-	-	2.50	-	-	-	45.00	17.50	-	-
Eupatorium riparium	1	7.14	16.70	4.76	4.76	-	-	16.66	35.70	14.28	-	-
	2	41.60	-	-	-	-	-	4.16	-	37.44	16.64	-
	3	-	10.32	-	-	-	-	27.52	20.64	24.08	17.24	-
Helianthus annuus	1	10.00	5.00	10.00	5.00	-	-	25.00	10.00	-	25.00	25.00
	2	25.90	7.40	-	-	22.20	7.40	14.80	7.40	-	-	14.80
	3	22.70	13.62	9.08	-	-	-	22.71	9.08	13.62	-	9.08
Pinus kesiya	1	31.21	-	-	-	-	-	15.60	24.98	-	15.60	12.48
	2	16.10	3.22	-	-	19.32	-	12.88	16.10	6.44	9.66	16.10
	3	9.09	-	6.06	-	-	-	9.09	36.36	6.06	21.21	12.12
Poultry litter	1	5.26	-	5.26	-	10.52	-	15.79	15.79	15.79	-	31.57
	2	28.00	-	-	-	-	-	12.00	16.00	12.00	12.00	20.00
	3	16.60	-	-	-	-	-	24.90	8.30	8.30	16.60	24.90
Control rhizosphere soil	-	6.25	-	-	12.50	-	-	3.12	49.93	-	18.72	9.36
Control soil	-	10.40	-	-	2.08	20.83	-	39.73	10.40	-	16.70	-

TABLE 2.9: Effect of organic amendments to soil on the percentage of relative abundance of rhizosphere fungi under infested condition.

Soil and rhizosphere fungi	Control (without amendment)	Control rhizosphere (without amendment)	Eupatorium adenophorum			Eupatorium riparium			Pinus kesiya			Helianthus annuus			Poultry litter		
			1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Absidia sp.	-	-	-	-	-	-	-	-	-	-	-	10.00	-	-	-	-	-
Aspergillus candidus Link ex. Fries.	-	-	-	-	-	-	16.64	-	-	-	-	-	11.10	-	-	-	-
A. clavatus Desm.	-	-	-	-	-	-	-	-	6.25	-	-	-	-	-	-	-	-
A. flavipes Thom & Church.	-	6.25	-	5.40	5.00	-	-	-	15.60	9.66	-	-	-	-	-	-	-
A. flavus Link ex. Fries.	10.40	-	5.12	-	5.00	7.14	4.16	-	-	6.44	3.03	10.00	-	-	5.26	4.00	-
A. fumigatus Fresenius.	-	-	-	-	-	-	-	-	-	-	6.06	-	-	-	-	12.00	-
A. nidulans (Eidem) Winter.	-	-	5.12	-	-	-	-	-	-	-	-	-	-	13.62	-	-	-
A. niger Van. Tieghem.	-	-	-	2.70	10.00	-	-	-	9.36	-	-	-	7.40	4.54	-	8.00	-
A. versicolor (Vuillemin) Tiraboschi.	-	-	-	-	15.00	-	20.80	-	-	-	-	-	7.40	4.54	-	4.00	16.60
Cephalosporium acremonium Corda.	-	-	-	8.10	-	16.66	-	10.32	-	-	-	-	-	9.08	-	-	-
Cephalosporium sp.	-	-	-	-	-	-	-	-	-	3.22	-	5.00	7.40	4.54	-	-	-
Cladosporium herbarum Link ex. Fries.	-	-	-	-	-	4.76	-	-	-	-	6.06	10.00	-	9.08	5.26	-	-
Fusarium oxysporium	2.18	12.50	5.12	8.10	2.50	-	-	-	-	-	-	5.00	-	-	-	-	-
F. solani	-	-	-	5.40	-	4.76	-	-	-	-	-	-	-	-	-	-	-
Geotrichum sp.	20.83	-	10.25	-	-	-	-	-	-	19.32	-	-	22.20	-	10.52	-	-
Gliocladium catenulatum Gilman & Abbott.	-	-	-	-	-	-	-	-	-	-	-	-	7.40	-	-	-	-
Humicola grisea Traaen.	20.83	-	-	-	-	-	-	-	-	-	-	-	11.10	-	-	-	-
Mucor circinelloides Van Tieghem.	-	-	-	-	-	11.90	-	-	-	3.22	-	-	-	-	-	-	-
M. hiemalis Wehmer.	18.90	-	-	2.70	-	-	-	13.76	-	-	-	-	-	9.08	-	-	-
Penicillium brefeldianum Dodge.	-	3.12	-	8.10	-	9.52	-	10.32	9.36	-	6.06	-	-	-	-	8.00	-
P. chrysogenum Thom.	10.40	6.25	12.80	10.80	10.00	9.52	-	10.32	6.25	-	12.12	10.00	-	9.08	5.26	4.00	-
P. granulatum Bainier.	-	3.12	10.25	-	10.00	-	-	-	6.25	-	6.06	-	-	-	-	-	-
P. oxalicum Thom.	-	-	-	-	5.00	14.28	-	-	-	12.88	-	-	-	-	-	-	-
P. rubrum Stoll.	-	18.72	10.25	21.62	15.00	2.38	-	-	-	-	12.12	-	-	-	-	-	8.30
P. vermiculatum Dangeard.	-	18.72	15.36	13.50	-	-	-	-	3.12	-	-	-	7.40	-	10.52	4.00	-
Penicillium sp.	-	-	-	10.81	5.00	-	-	-	-	3.22	-	-	-	-	-	-	-
Pythium sp.	-	-	5.12	-	-	-	-	-	-	6.44	-	-	7.40	-	-	-	-
Rhizopus stolonifer (Ehrenb ex. Link). Lind 1913.	-	3.12	-	2.70	-	4.76	4.16	13.76	15.60	9.66	9.09	15.00	3.70	13.62	15.79	12.00	24.90
Trichoderma harzianum Rifai.	-	-	-	-	-	4.76	20.80	13.76	-	-	-	-	-	-	15.79	12.00	-
T. koningii oudemans.	-	-	2.56	-	10.00	9.52	16.64	10.32	-	6.44	6.06	-	-	-	-	-	-
T. viride Pers. ex. Fries.	-	-	-	-	7.50	-	-	-	-	-	-	-	-	13.62	-	-	8.30
Yeast (red)	16.70	18.72	12.80	-	-	-	16.64	17.25	15.60	9.66	21.21	25.00	-	-	-	.12.00	16.60
Unidentified	-	9.36	5.12	-	-	-	-	-	3.12	9.66	12.12	10.00	7.40	9.08	31.57	20.00	24.90

Amendments applied to soil at a rate of 1, 2, 3% w/w.

Disease symptom was found to appear in the cotyledons at the time when the seedlings were 3 weeks old. The cotyledons gets detached from the seedlings following the infection. The visual record of infection at the foot region of soybean seedlings observed on the fifth week of age. Reduction in infection to plant was observed with all the amendments compared to non amended infected control. Highest reduction was recorded with **E. riparium** (3%w/w) amendment (12.2% infection). With the increase in concentration of the leaf material amendment to soil, a reduction in the disease severity was observed in most of the cases (Plate 3,4). Concentration of poultry litter showed a direct relationship to the severity of the disease, as an increase in disease severity was observed with the increase in the concentration of the poultry litter applied (Table 2.10). Some of the soil amendments delayed the symptom expression, together with the reduced infection rate, possibly due to the slow release of the inhibitory substances or rapid stimulation of the antagonists in soil. **Eupatorium riparium** amendment to soil, delayed the symptom expression as well as reduced the disease severity possibly through the stimulation of the antagonistic **Trichoderma** spp. in the soil (Fig. 4.2B). Although **E. adenophorum** showed an increase infection rate at the initial stage, compared to other treatment, the rate slowed down with time, probably due to the slow release of inhibitory substances in the soil (Fig. 4.2A). Other soil amendments i.e. **Pinus kesiya, Helianthus annuus** and poultry litter, also released

Plate 3: Showing the effect of soil amendments on the foot rot symptom/disease control and the growth of soybean plants compared to control (infected): i) **Eupatorium adenophorum** and (ii) **Eupatorium riparium** (control, 1, 2, 3% w/w of organic amendments from left to right).

PLATE-3

(i)

(ii)

Plate 4: Showing the effect of soil amendments on the foot rot symptom/disease control and the growth of soybean plants compared to control (infected): i) **Helianthus annuus** and (ii) Poultry litter (control, 1,2,3% w/w of organic amendments from left to right).

PLATE-4

(i)

(ii)

TABLE 2.10 : Effect of poultry litter on the disease development of soybean plant.

Amendments	Concen-tration (%)	Percentage of infection/week									
		5	6	7	8	9	10	11	12	13	14
Poultry litter	1	7.14	10.70	12.50	17.86	19.64	19.64	25.00	28.57	30.36	35.70
	2	7.55	9.43	13.21	13.21	16.98	18.87	22.64	28.30	32.08	37.74
	3	9.80	9.80	19.60	21.56	27.45	31.37	31.37	33.30	39.21	43.13
Infected control (without amendment)	-	15.40	25.00	30.80	32.70	32.70	36.50	38.50	46.20	63.50	71.20

Fig. 4.2 Effect of organic amendments (i.e. A = **Eupatorium adenophorum,** B = **Eupatorium riparium,** C = **Pinus kesiya,** D = **Helianthus annuus**) on the disease development of soybean caused by **Sclerotium rolfsii.**

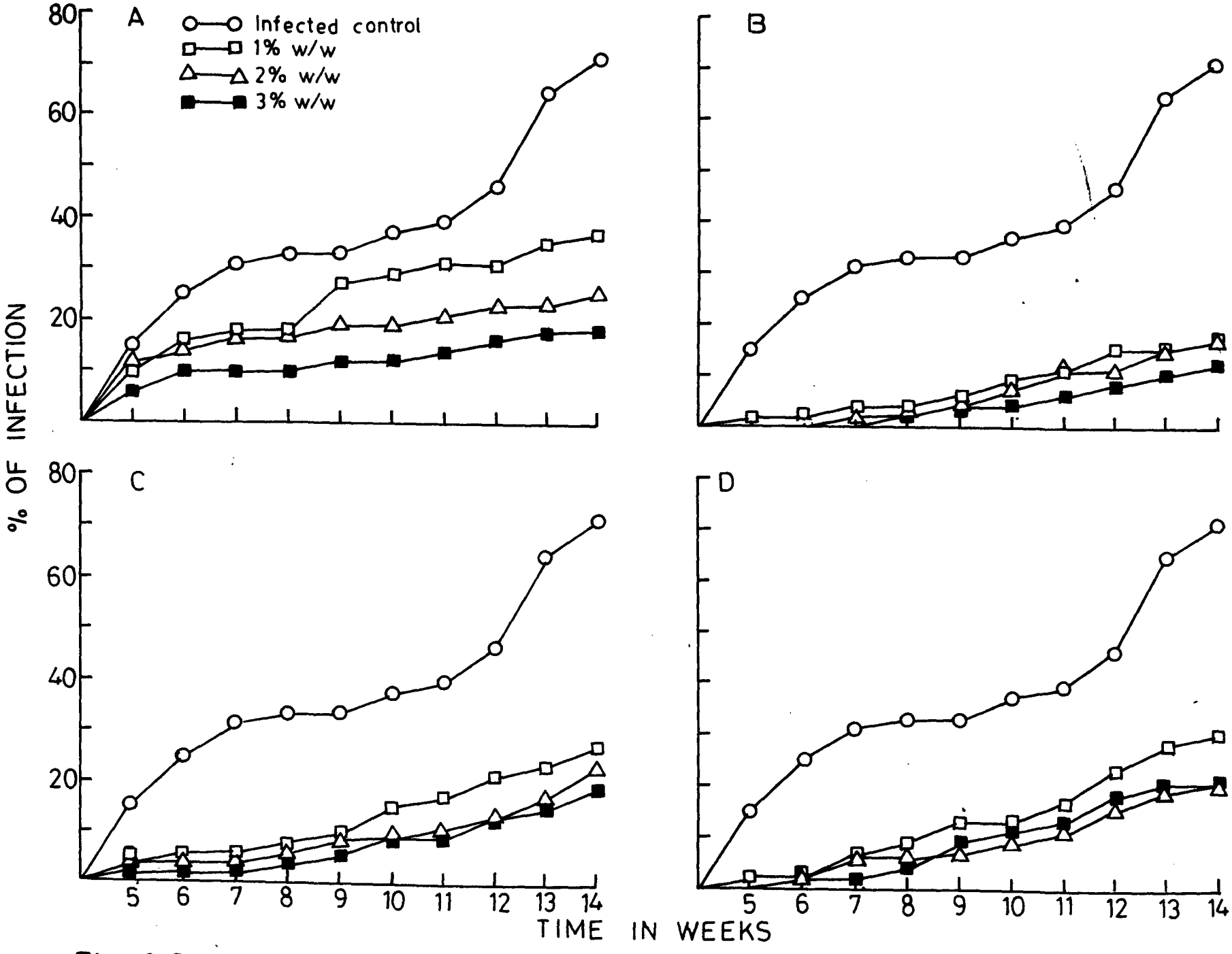

A
B
C
D
Infected control
1% w/w
2% w/w
3% w/w
% OF INFECTION
TIME IN WEEKS
80
60
40
20
5
6
7
8
9
10
11
12
13
14

Fig. 4·2

toxic/inhibitory substances into the soil, resulting in a decrease of the disease severity (Fig. 4.2C,D). In general, almost 50% reduction in disease severity was achieved with all the amendments compared to non amended infected control.

Population dynamics of S. rolfsii in amended soil

Gradual increase in **S. rolfsii** population was observed in the non amended pathogen infested soil, while in amended soil, **S. rolfsii** population found to increase at the initial stage (observed after 30 days) excepting in the soil amended with **E. adenophorum** and **E. riparium** but subsequently, a gradual reduction in population was recorded in all the cases (Fig. 4.3). This increase in population at the initial stage is probably due to the breaking of imposed dormancy of resting structure (i.e. **S**clerotia) and their saprophytic colonisation. Poultry litter and **Helianthus annuus** amendments showed higher increase in **S. rolfsii** population, which later reduced considerably (Fig. 4.3E,C). In the **E. adenophorum** amended soil, the population remained almost static (Fig. 4.3A) whereas in **E. riparium** amended soil, an abrupt increase in population was also recorded at the time of second sampling period (i.e. after 60 days) which subsequently declined sharply. (Fig.4.3B).

Effect on plant height

The soil amendments did not show any adverse effect on seedling height excepting **Helianthus annuus** and poultry

Fig. 4.3: Effect of organic soil amendments (i.e. A. **Eupatorium adenophorum,** B. **Eupatorium riparium,** C. **Helianthus annuus,** D. **Pinus kesiya,** E. Poultry litter) on the population dynamics of **Sclerotium rolfsii** in natural soil. (vertical bar represent LSD at P=0.05 level).

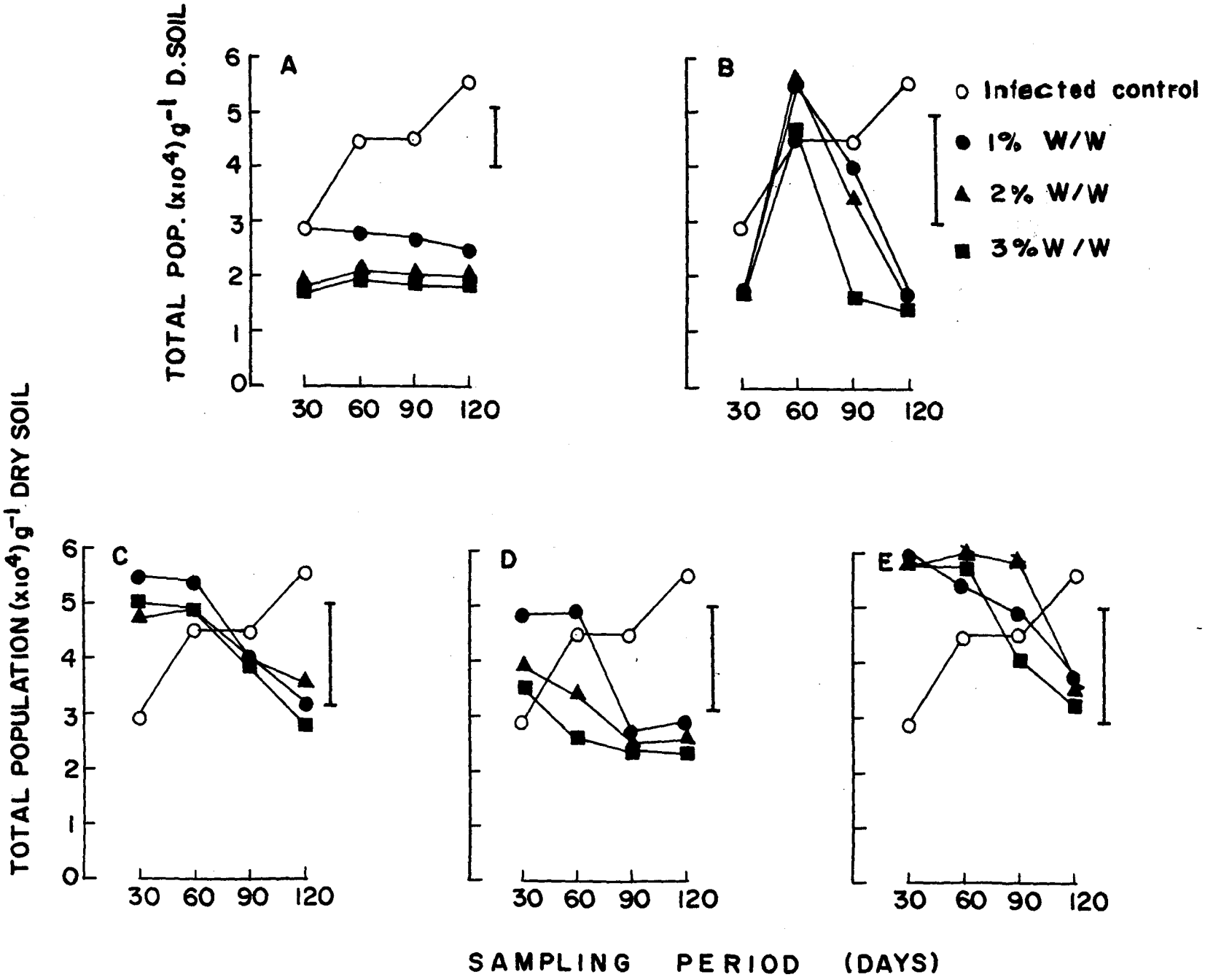

TOTAL POP. (x10⁴) g⁻¹ D.SOIL
TOTAL POPULATION (x10⁴) g⁻¹ DRY SOIL
A
B
C
D
E
0
1
2
3
4
5
6
30 60 90 120
Infected control
1% W/W
2% W/W
3% W/W
SAMPLING PERIOD (DAYS)

Fig. 4·3

litter amendments (Table 2.11). The plants attained their maximum height when they were ten weeks old. Higher concentration of poultry litter (i.e. 3% w/w) was found to be slightly toxic as it showed yellowing of leaves. Lower concentration (i.e. 1% w/w) favoured the growth and development of soybean plants. **Helianthus annuus** was also found to be suitable for the growth of soybean plants. These two soil amendments increased the plant height (Plate 4), leaf area and yield, compared to non amended infected control.

Dry matter accumulation and yield

Dry matter accumulation of soybean plants (i.e. dry weight of root, shoot and pod) increased due to the soil amendments in all the cases compared to non amended control. Increased plant height resulting in an increased dry weight of the shoot/plant was observed following poultry litter (9.0g/plant in case of 3% w/w) and **H. annuus** (8.5g/plant in 1% w/w) amendment. Poultry litter amendment also increased the dry weight of the root (3.1g/plant at 2% w/w). Increased pod weight was recorded in case of **E. riparium** (3% w/w) (10.0g/plant) and poultry litter (2% w/w) amendment (10.8g/plant) (Table 2.12).

Soybean yield also increased in all the treatments compared to non amended infected control. **E. riparium** (3% w/w) and poultry litter (2% w/w) amendments to soil, increased yield 9.0g and 9.33g/plant, respectively (Table 2.12). **Pinus kesiya** needle amendment to soil reduced the dry matter accumulation

TABLE 2.11 : Effect of organic soil amendments on the growth (Plant height in cm)[1] of soybean plant.

Amendments	Concentration(%)	Time in weeks									
		1	2	3	4	5	6	7	8	9	10
Eupatorium adenophorum	1	9.14 ±0.33	14.05 ±0.54	16.20 ±0.56	18.40 ±0.73	22.30 ±0.84	28.80 ±0.94	36.00 ±1.18	40.81 ±2.75	47.40 ±2.42	52.67 ±2.05
	2	10.50 ±0.30	13.00 ±0.65	16.60 ±0.44	18.60 ±0.71	22.70 ±0.93	30.87 ±0.83	35.70 ±0.97	45.00 ±1.09	49.36 ±1.19	53.10 ±1.00
	3	11.00 ±0.36	13.50 ±0.53	15.08 ±0.51	19.10 ±0.96	23.40 ±0.97	32.40 ±0.94	39.00 ±1.39	52.56 ±1.30	53.55 ±1.68	57.10 ±1.09
Eupatorium riparium	1	10.00 ±0.25	12.00 ±0.71	16.50 ±0.64	18.42 ±0.70	26.31 ±1.19	27.93 ±1.16	36.67 ±1.10	41.25 ±1.65	46.27 ±1.92	52.81 ±1.18
	2	12.20 ±0.51	15.54 ±0.68	19.69 ±0.67	21.76 ±0.68	28.00 ±1.10	31.00 ±0.64	37.30 ±1.14	42.80 ±1.04	47.42 ±1.79	53.20 ±1.52
	3	10.54 ±0.32	14.00 ±0.51	16.89 ±0.69	20.00 ±0.47	28.06 ±1.15	31.96 ±1.16	39.10 ±1.73	43.09 ±2.43	47.41 ±2.28	54.72 ±0.98
Helianthus annuus	1	14.20 ±0.42	17.50 ±0.38	19.15 ±0.47	20.30 ±0.42	29.13 ±0.75	35.40 ±0.98	41.67 ±1.57	55.92 ±2.29	56.72 ±2.29	66.20 ±2.45
	2	11.09 ±0.36	14.35 ±0.61	17.62 ±0.37	22.66 ±0.38	30.06 ±0.77	36.40 ±1.14	42.75 ±0.65	60.18 ±1.04	65.10 ±2.97	72.40 ±2.74
	3	13.00 ±0.41	16.10 ±0.79	17.43 ±0.35	21.80 ±0.65	40.20 ±1.13	41.10 ±1.88	46.63 ±1.51	62.08 ±1.16	69.16 ±2.00	79.60 ±1.57
Pinus kesiya	1	12.10 ±0.57	13.00 ±0.87	16.70 ±0.61	19.16 ±0.54	25.20 ±0.73	32.33 ±0.65	35.30 ±0.69	40.75 ±1.86	47.08 ±1.30	51.63 ±1.90
	2	11.45 ±0.49	14.20 ±0.93	16.66 ±0.59	18.48 ±0.66	23.40 ±0.91	26.86 ±1.30	30.18 ±2.16	34.60 ±0.77	43.50 ±2.24	49.54 ±1.17
	3	11.20 ±0.63	12.30 ±0.83	15.62 ±0.73	19.48 ±0.53	26.06 ±1.06	31.73 ±1.15	37.00 ±1.10	41.55 ±1.13	46.09 ±1.87	46.22 ±2.39
Poultry litter	1	14.90 ±0.81	16.20 ±0.77	19.45 ±0.55	22.40 ±0.41	28.00 ±1.59	39.33 ±0.79	45.60 ±2.28	56.50 ±3.70	69.30 ±1.88	78.00 ±3.44
	2	15.50 ±0.45	17.00 ±0.89	20.84 ±0.92	32.95 ±0.66	39.70 ±2.16	45.93 ±1.78	59.20 ±1.47	68.87 ±3.72	78.60 ±1.96	83.22 ±1.19
	3	13.29 ±0.68	18.10 ±0.77	24.35 ±0.59	30.04 ±0.59	40.73 ±2.10	48.80 ±1.17	55.50 ±2.04	64.70 ±2.21	70.25 ±3.07	72.76 ±2.87
Infected control (without amendment)	-	7.50 ±0.88	11.35 ±0.76	15.19 ±0.40	17.10 ±0.76	22.07 ±1.35	24.06 ±0.95	37.90 ±1.70	40.97 ±2.28	47.50 ±2.04	51.00 ±2.79

1) Mean of 30 replicates with ±S.E.

TABLE 2.12 : Effect of Organic soil amendments on the dry matter accumulation and yield of soybean plants.[1]

Amendments	Concentration(%)	Dry weight of shoot/plant (gm)	Dry weight of Root/Plant (gm)	Pod weight/ Plant (gm)	Yield/ Plant (gm)
Eupatorium adenophorum	1	7.00	2.00	7.50	6.50
	2	7.70	2.25	6.18	5.43
	3	8.00	2.50	7.12	6.83
Eupatorium riparium	1	7.62	2.20	7.25	6.13
	2	7.40	2.70	6.90	5.88
	3	7.85	2.90	10.00	9.00
Helianthus annuus	1	8.50	2.50	8.80	7.00
	2	7.00	2.50	8.05	7.38
	3	7.00	2.75	8.05	6.43
Pinus kesiya	1	7.80	2.00	6.00	5.25
	2	6.00	2.30	7.50	5.50
	3	5.50	1.90	6.20	5.00
Poultry litter	1	7.50	3.00	7.80	6.40
	2	8.00	3.10	10.80	9.33
	3	9.00	3.00	8.65	7.13
Infected control (without amendment)	-	5.00	2.50	5.00	4.00
LSD(P=0.05)	-	1.65	0.39	2.44	2.33

1) Mean of 30 plants in each case.

of root, shoot and yield compared to other amendments.

Experiment under field condition

Estimation of S. rolfsii population in experimental field

It was observed that the population of **Sclerotium rolfsii** increased immediately after soil amendments, in general. Mycelial mats of **S. rolfsii** was observed when the soil received first shower after the amendments. Higher concentration of the amendments (40q/ha), reduced the pathogen's (i.e. **S. rolfsii**) population compared to lower concentration (i.e. 10q/ha). **S. rolfsii** population also decreased with time in the amended soil, considerably (Fig. 4.4).

Disease severity

Under field trial, infection symptom of the seedlings observed when they were one week old, resulting in death. The rate of infection/plot increased slowly compared to unamended infected control. Higher concentration (40q/ha) of **E. adenophorum** and **E. riparium** leaf amendment to soil, reduced the severity of the disease to a great extent (22.2% and 21.95% infection respectively), (Table 2.13) compared to infected non amended control (65.85%) (Plate 5,6). In general, a reduction in disease severity was observed with all the soil amendments used.

Soybean plant height

Little variation in plant height was recorded till

Fig. 4.4: Effect of organic soil amendments (A. **Eupatorium adenophorum**, B. **Eupatorium riparium**) on the population dynamics of **Sclerotium rolfsii** under field condition (vertical bar represent LSD at P=0.05 level).

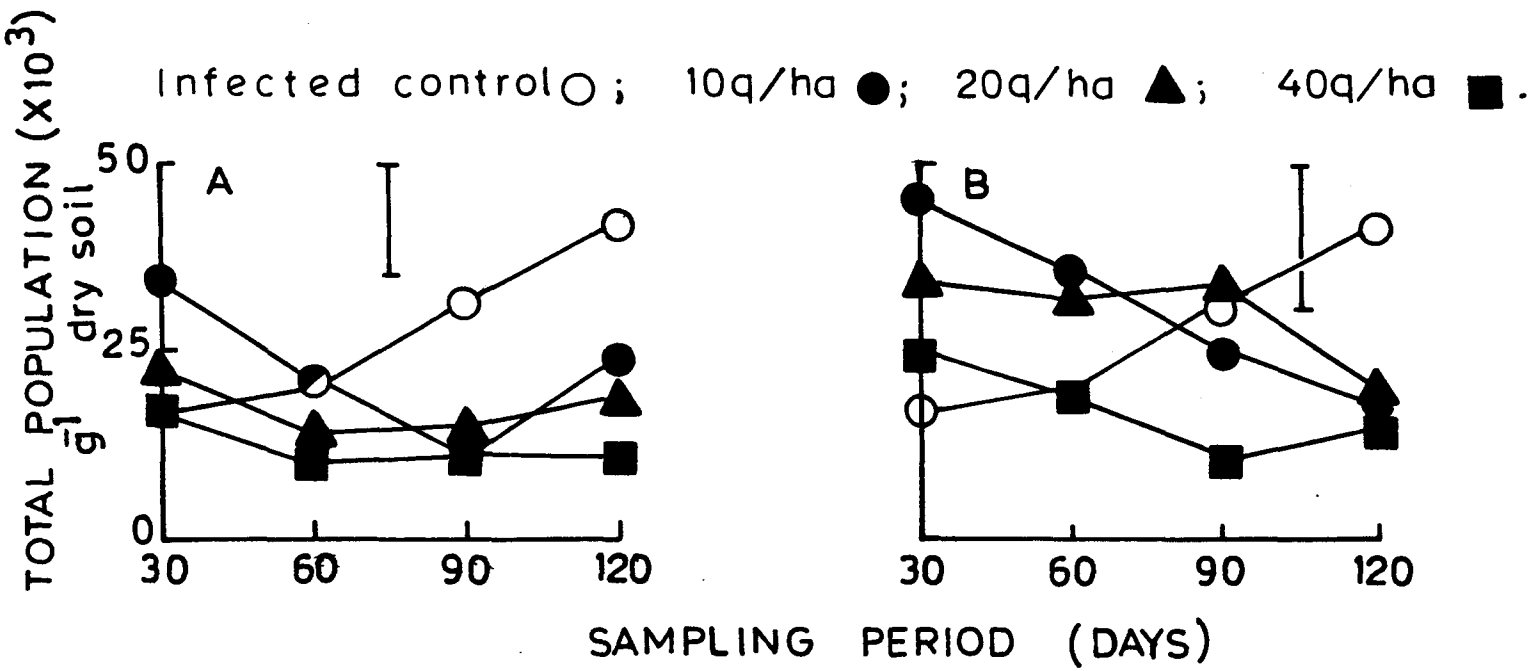

Infected control ○ ; 10q/ha ● ; 20q/ha ▲ ; 40q/ha ■ .
TOTAL POPULATION (X10³) g⁻¹ dry soil
50
25
0
A
B
30
60
90
120
SAMPLING PERIOD (DAYS)

FIG. 4·4

TABLE 2.13 : Effect of Organic soil amendments on the height[1] of soybean plants under field condition (values in Parenthesis indicate percentage of infected plants[2]).

Amendments	Concentration (q/ha)	Time in weeks				
		2	4	6	8	10
Eupatorium adenophorum	10	13.00±0.36 (10.10)	23.70±0.95 (12.12)	58.13±2.10 (15.56)	77.99±3.28 (30.00)	99.70±2.28 (30.30)
	20	15.84±0.31 (8.25)	24.30±0.91 (10.60)	61.97±1.54 (15.75)	86.25±4.42 (17.00)	97.64±2.31 (22.70)
	40	11.69±0.41 (7.58)	19.13±0.42 (11.43)	53.55±1.23 (16.40)	69.30±2.45 (19.40)	76.97±1.53 (22.20)
Eupatorium riparium	10	12.00±0.32 (9.25)	23.50±0.65 (12.33)	57.06±1.78 (17.60)	85.20±4.95 (26.20)	104.00±2.87 (35.70)
	20	14.00±0.34 (6.00)	23.06±0.76 (11.45)	59.57±1.77 (19.10)	76.75±4.67 (23.65)	98.06±2.74 (34.80)
	40	9.50±0.57 (7.95)	19.48±0.63 (10.50)	36.40±0.85 (16.13)	47.40±2.79 (17.86)	61.01±1.40 (21.95)
Infected control (without amendment)	-	11.00±0.40 (22.00)	18.10±1.01 (36.58)	52.95±1.25 (43.33)	75.06±4.34 (54.00)	87.04±1.45 (65.85)

1) Mean of 10 plants with ±S.E.
2) Calculations based on 100 plants (approx.)/plot (5 sq.m) in each case.

Plate 5: Photograph showing foot rot disease of soybean caused by **Sclerotium rolfsii** under field condition.

PLATE-5

Plate 6: Showing reduction in disease severity of soybean achieved by (i) **Eupatorium adenophorum** and (ii) **Eupatorium riparium** (40q/ha) amendments to soil.

PLATE-6

(i)

(ii)

the plant obtained their maximum height (Table 2.13). It was observed that the higher concentration (40q/ha) of organic amendments are slightly inhibitory to the plant growth (Plate-7). Lower concentration of **E. adenophorum** (10q/ha) and **E. riparium** (10q/ha) increased the plant height to the extent of 99.7cm and 104cm respectively, compared to non amended infected control (87.04cm).

Yield

Soybean seed yield in the amended soil was always higher compared to the infected non amended control plots. Highest yield (12.12 q/ha) was recorded with **E. adenophorum** (40q/ha) followed by **E. riparium** (8.7q/ha) amendment (Fig. 4.5). In general with the increase in concentration of the amendments, an increase in the yield was also observed.

DISCUSSION

Most of the leaf extracts suppresed the growth of **S. rolfsii in vitro. Eupatorium adenophorum** and **E. riparium** leaf extract gave maximum inhibition in growth of **S. rolfsii** compared to stem and root, (result not presented in this thesis) suggested that the inhibitory substances were accumulated more in the leaves. There was a negative correlation between the concentration of the extract used and the growth of **S. rolfsii**

Plate 7: Showing slight reduction in the growth of soybean plants following higher amount of (i) **Eupatorium adenophorum** and (ii) **Eupatorium riparium** amendments (40 q/ha) to soil.

PLATE-7

(i)

(ii)

Fig. 4.5: Effect of organic soil amendments on the yield of soybean under field condition.

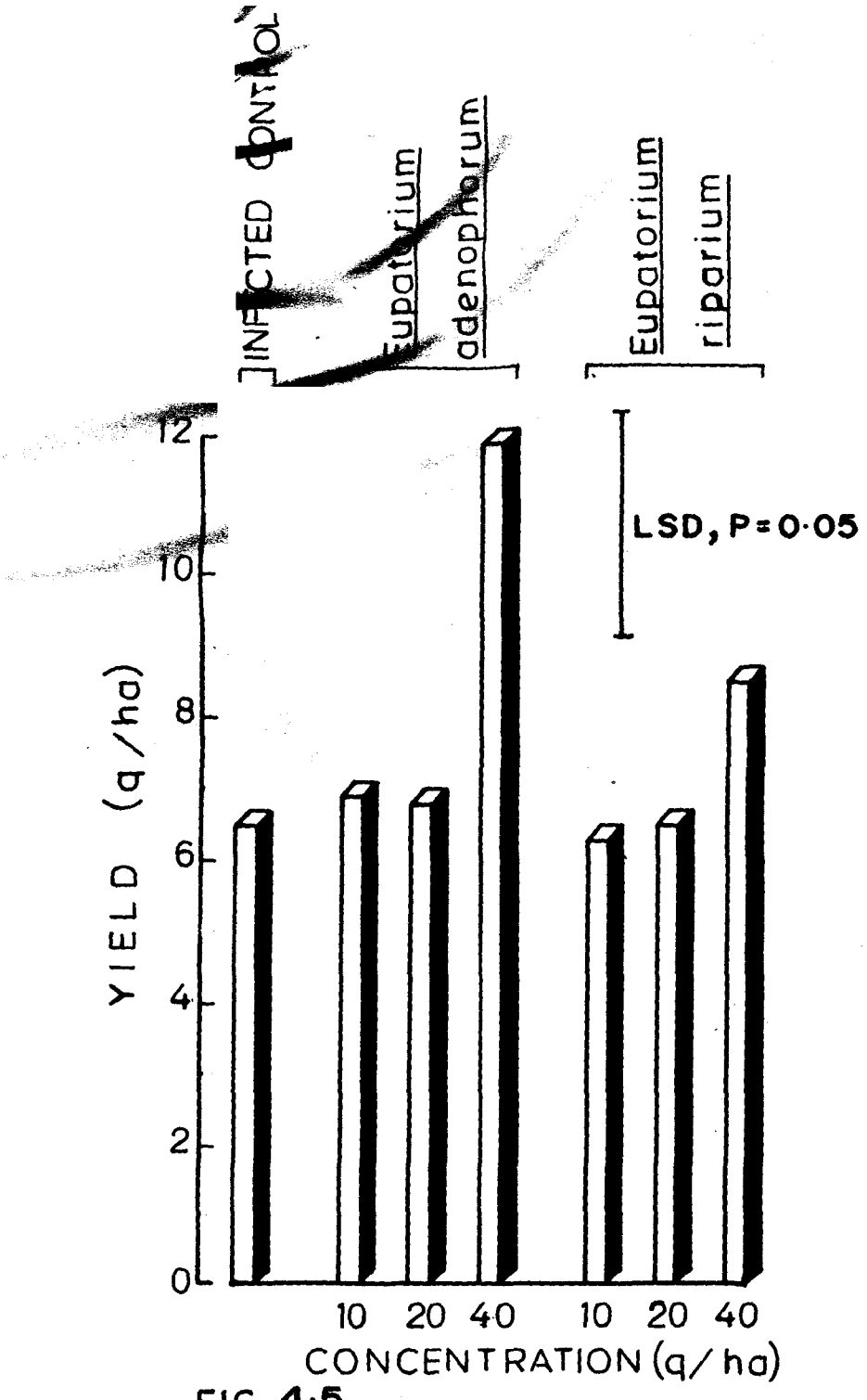

INFECTED CONTROL
Eupatorium adenophorum
Eupatorium riparium
LSD, P=0·05
YIELD (q/ha)
0
2
4
6
8
10
12
10 20 40 10 20 40
CONCENTRATION (q/ha)

FIG. 4·5

as observed with the above mentioned plant materials. **Pinus kesiya** and **Helianthus annuus** showed slight inhibition (nearly 20% at higher concentration). All the plant materials used in the present work inhibited the growth of the test pathogen, suggesting that the inhibitory substances present were mostly water soluble. Other workers (Schmidt, 1955; Seikel, 1964; Barlocher and Oertli, 1978) have reported that the water soluble inhibitors from plants are possibly phenolic compounds.

Very little is known about the survival ability of sclerotium of **S. rolfsii** in natural soil, and the factors involved for their survival and germination. Some studies were conducted by Abeygunawardena and Wood (1957b), mostly with sterilised soil, which was found to have no relevance to field conditions. Williams and Western (1965) suggested that the sclerotia remain viable in the natural soil for two years although they are known to survive for five years in tube culture (Povah, 1927). In our present observation although sclerotia remained viable upto 60 days in most of the amended soil but the percentage of viability was found to be very less. In case of **Helianthus annuus** and poultry litter (3%w/w) amendments the viable sclerotia was completely eliminated from the soil on 60 days where empty sclerotial shell only were recovered. Maiti (1977) also recorded the viability of sclerotia under controlled temperature and humidity condition, for 8 months, after which a gradual fall in the survibality

was observed.

The leaf extract had no apparent affect on the germinability of sclerotia, when incorporated directly to the sterilised medium. **E. adenophorum** (3% v/v) and **Pinus kesiya** (3%v/v) only produce mild inhibitory effect. When the sclerotia directly incubated in water extract of organic materials, higher concentration of the same showed inhibition in germination. The possible release of inhibitory substances (volatile and non volatile) through biological degradation of **E. adenophorum** may possibly impose dormancy to the sclerotia, as a result they remain dormant but viable and once they are placed in the suitable media they showed germination. The exudation of nutrients from the sclerotia may increase microbial antagonism and accelerate death of the organisms (Coley-Smith **et al**, 1974). In the present study, loss of viability of sclerotia in soil after 30 days in case of **E. riparium** and **Pinus kesiya** needle amendments, were possibly through microbial antagonism and higher accumulation of non volatile and volatile active compounds in the soil.

Although, **E. adenophorum** and **E. riparium** leaf extracts suppressed the seed germination and radicle growth of various weeds (Tripathi **et al**, 1981; Rai and Tripathi, 1984) but it did not produce any adverse affect on soybean seed germination and radicle growth indicates that the plant materials could

be used for plant growth.

It is known that the soil amendment changes microbial population in soil and rhizosphere. Some of the amendments in the present work have been found to be stimulatory, while some showed inhibitory effect i.e. **E. riparium** amendment increased the fungal population vigorously in soil and rhizosphere. Generally the addition of organic matter to soil increases the population of the soil saprophytes and thus becomes instrumental to suppress the disease organisms. Organic amendments mostly found to increase the fungal population in the soil but a reduction of the same have been observed in the rhizosphere, possibly due to changed root exudate pattern, thereby affecting the rhizosphere microflora in the present study. It was also reported earlier that **E. adenophorum** and **E. riparium** amendment to soil release toxins during the decaying process and produce detrimental affect on the soil microflora (Rai and Tripathi, 1984). In addition to fungal population, bacterial (excepting **E. adenophorum** and **E. riparium** amendment) and actinomycete population found to have increased in soil, suggesting that the disease control was achieved due to suppression of **S. rolfsii** and/or due to the biological activity of microbes in the soil. Kundu and Nandi (1985) reported that the soil amendments viz. commercial cellulose, rice stubbles or water hyacinth in combination with NH_4NO_3, increased the total microbial population together with the organisms antagonistic to the test pathogen (i.e. **Rhizoctonia solani**).

Penicillium spp., **Aspergillus** spp. and mucorales found to be present in all the amended soil suggests their rapid growth and colonization over the leaf material which suppressed **S. rolfsii** in the soil. Stimulation of **Trichoderma** spp. i.e. **T. harzianum** and **T. koningii** and **Penicillium** spp. due to **E. riparium** and **Penicillium** spp. only due to **E. adenophorum** soil amendments, possibly reduce the inoculum potential of **S. rolfsii** in soil and subsequently a reduction in disease severity occurred. **Penicillium** spp. **Aspergillus** spp. and **Trichoderma** spp. were also found to be dominant in the rhizosphere of soybean following soil amendments.

Seed rot due to **S. rolfsii** in soil following organic amendment was found to be more and is due to its high competitive saprophytic ability (Sengupta and Roy, 1971) in the initial stage of biodegradation. Monthly record of **S. rolfsii** population revealed that the rapid build up of inoculum density due to soil amendment resulted the onset of infection of seedlings within one month in the field trial but to a slower rate in the pot experiment (i.e. symptom appeared after 5th week of amendments). **E. adenophorum** amendment suppressed the **S. rolfsii** population with a sustained effect throughout the cropping season (in pot experiments).

It has been reported earlier that the dead organic substrate in soil provides energy for infection to the sclerotia of **S. rolfsii** (Boyle, 1961). In the present observation, energy

provided by soil amendments stimulated the antagonistic micro-organisms causing infection to **S. rolfsii**. Other organic amendments showed a rapid increase in **S. rolfsii** population in soil within 30 days after which it declined and thus reduced the disease intensity. Similar observation was also made by Mixon (1965), Linderman and Gilbert (1969) that the crop residue stimulated sclerotial germination followed by lysis of mycelium and increased colonization of sclerotia by soil antagonists, saprophytes and consequently, reduced the inoculum density. Survival of sclerotia in soil was also reported to be reduced by amending the soil with alfalfa hay (Johnson, 1953; Menzies and Gilbert, 1967). Exposure of soil to vapours of alfalfa distillate also reduced the viable sclerotia. Stimulation of mycelial growth from sclerotium when exposed to alfalfa distillate in natural soil, was observed by Linderman and Gilbert (1973a,b). Soil microbial population and respiration studies indicated that the growth inhibition of **S. rolfsii** may be related to the increased activity of soil microbes, primarily bacteria. In the present study an increase in microbial population viz. fungi, actinomycete and bacteria (excepting **E. adenophorum** and **E. riparium**) could have inhibited the growth and multiplication of **S. rolfsii** in soil, through the microbial antagonism and possibly through the release of inhibitory substances during decomposition (in case of **E. adenophorum** and **P. kesiya**) of the organic amendments causing a reduction in pathogen's population and disease severity.

The infection rate in soybean plant was found to be rather slow in the amended soil compared to non amended infected control. **E. riparium** delayed symptom expression possibly due to the decrease in inoculum density of **S. rolfsii** by the increased **Trichoderma** spp. population in soil. **Pinus kesiya** and **Helianthus annuus** reduced the disease at higher concentration while lower concentration of poultry litter was found to be suitable for plant growth. Dutta and Isaac (1979 a,b) correlated the decrease of disease severity with the increase in the population of actinomycete in the rhizosphere. In the present work, reduced infection rate in the amended soil is probably due to the combined effort of antagonistic soil/rhizosphere microflora and the inhibitory substances released during decomposition process in soil. Population of rhizospheric bacteria increased after soil amendments resulting in reduction of disease severity which may be correlated with the view of Linderman and Gilbert (1973 a,b) who also observed an increased inhibition in sclerotial growth by the increased activity of soil bacteria.

Organic amendments to soil in the present study suppressed the total fungal and actinomycete population but stimulated the **Penicillium** spp., **Trichoderma** spp. and bacteria, which might have implication in the control of foot rot of soybean caused by **S. rolfsii.**

The dominant weed species, **E. adenophorum** and **E. riparium** widely known as noxious ruderal weed of Meghalaya grows luxuriantly

on the waste lands and road sides show a positive control of **S. rolsfii in vitro** and **in vivo** when applied as soil amendment. Increase in dry matter accumulation and soybean yield after organic amendments also suggests the possibility of using these amendments for the control of the foot rot disease. Poultry litter gave better yield (at 2% w/w) and dry matter accumulation supports Arora and Takkar's (1981) findings, where they found that the poultry manure contains higher amount of N and Ca, resulting increased uptake of all the nutrients and also increased the dry matter and yield of wheat.

The green leaf manure known to liberate more nitrogen than that of dry under high temperature and moisture resulting in increased yield, probably due to increased uptake of nitrogen and calcium as reported by Chatterjee **et al** (1979). In the present study, **E. adenophorum** and **E. riparium** (green leaf only) at a rate of 40q/ha under field trial reduced disease severity to a great extent together with increased yield corroborates this view.

CHAPTER III

EFFECT OF INORGANIC AMENDMENTS ON THE SOIL AND RHIZOSPHERE MICROFLORA IN RELATION TO THE BIOLOGY AND CONTROL OF SCLEROTIUM ROLFSII CAUSING FOOT ROT OF SOYBEAN

INTRODUCTION

Effect of inorganic amendments on the soil microbial population are well known. Microorganisms, breaks the complex inorganic substances into simpler form and makes it available to plants. Treatment of soil with fertilizers directly influences its microbial population and it was reported earlier that addition of N,P and K to soil at certain concentration stimulate dormant genera of fungi without affecting other soil inhabitants (Waksman, 1922). With the exception of lime and sulphur, inorganic amendments are applied at a much lower rate, before sowing and also during the growing season of the plant. The effect that inorganic amendments have on plants are either immediate without mediation of soil microorganisms or indirect during biological transformation.

Effect of nitrogenous fertilisers on the disease caused by pathogen has been reported by several workers (Leach and Davey, 1942; Mohr and Watkins, 1959; Ranney, 1962; Lapwood and Dyson, 1966; Sulaiman and Rhode, 1968; Erinle and Edmunds, 1970; Thakur and Mukhopadhyay, 1972; Punja and Grogan, 1982; Punja **et al**, 1985). In some cases, an increased disease severity have been reported (Walker and Foster, 1946; Dorworth and Tammen, 1969; Erinle and Edmunds, 1970; Huber, 1972; Sivaprakasam and Rajagopalan, 1974), while at the same time decreased in disease severity has also been observed in some cases (Leach and Davey, 1942; Zentmyer and Bingham, 1956; Sequeira, 1963;

Maurer and Baker, 1965; Sulaiman and Rhode, 1968; Thakur and Mukhopadhyay, 1972). Reduction in severity caused by **S. rolfsii** following applications of anhydrous ammonia (Leach and Davey, 1935, 1942), ammonium sulphate (Leach and Davey, 1942), ammonium nitrate (Mc. Clellan, 1947) and calcium nitrate (Leach and Davey, 1942; Sitterly, 1962) has also been reported. It was observed that the severity of **Verticillium** wilt of cotton was increased two folds by ammonium or nitrate nitrogen as compared with ammonium nitrate (Ranney, 1962). Dutta and Isaac (1979a) reported that the addition of ammonium sulphate (0.25%), calcium nitrate (0.25%, 0.5%) and combined NPK (0.25%) to soil caused considerable reduction in wilt disease of antirrhinum caused by **Verticillium dahliae.** It was further observed by them that the ammonium sulphate and combined NPK (0.25%) in soil also delayed the natural senescence in healthy plants.

Zinc deficiency is common in most of the Indian soil and has been observed to be associated with the introduction of high yielding varieties of crops and intensive cropping system. The response of various crops to zinc deficiency in soil has been reviewed by Kanwar and Randhawa (1974). Phosphorus and zinc, the important macro and micronutrient, plays a dominant role in yield and disease incidence. The disease incidence has been observed to be negatively related to zinc but positively with phosphorus applied to soil (Singh and Aggarwal, 1979). Zinc is also reported to increase the crop yield as

observed by Kanwar (1964) and Takkar **et al** (1971), in case of wheat. Dutta and Bremner (1981) reported that the root dip application of zinc sulphate (10 ppm) gave reasonably good control of the wilt disease of tomato caused by **Verticillium albo-atrum R & B.**

Liming (combination of hydrated lime and ground lime stone) increased the soil pH, possibly due to immobilization of micro nutrients (Jones and Woltz, 1967, 1969, a,b) resulting reduction in incidence and severity of **Fusarium** wilt of tomato (Jones and Woltz, 1970).

Much importance has been given to the changes in disease incidence or severity due to the application of inorganic amendments or due to adjustment of soil reaction, results from the effects on each of the living components that are involved in the disease i.e. the pathogen, host and soil micro-organisms. The amendments may be beneficial to the pathogen or can increase the host susceptibility, but in most of the cases their application still results in the reduction in disease incidence or severity. While the reaction of plants to the addition of fertilisers has received attention, little information is available regarding the effect of mineral nutrients to the rhizosphere microflora in relation to disease development. Therefore, an attempt has been made to study the effect of inorganic soil amendments on the rhizosphere microflora of soybean plants grown in **Sclerotium rolfsii** i[illegible]d.

soil with special reference to disease development and control.

MATERIALS AND METHODS

Studies on the effect of inorganic amendments in relation to the growth of **S. rolfsii in vitro** and **in vivo** includes few inorganic fertilisers i.e. calcium carbonate, calcium nitrate, urea, ammonium nitrate, rock phosphate and zinc sulphate.

Since for our convenience, we categorised all the green plant materials and poultry litter as organic fertilisers therefore, we have placed urea in inorganic amendment chapter. To observe the effect of amendments, the fungus was grown on PDA amended with different concentrations (i.e. 0.1, 0.25, 0.5, 1.0% w/v) of the inorganic fertilisers. Sclerotial germination in PDA amended with inorganic chemicals and survibality of sclerotium in aqueous solution was done, following direct observation and also by Agnihotri **et al** (1975) method as described in page 41 .

Survibality of Sclerotium in the amended soil in vitro

Inorganic chemicals at different concentrations (0.1, 0.25, 0.5% w/w) were mixed thoroughly to the soil and was kept in plastic pots for 7 days. Subsequently, 30 sclerotia were mixed properly with the amended soil and was incubated as described earlier (page 41). The sclerotia recovered

in 15 days interval and their germinability was determined following the method described by Agnihotri **et al** (1975).

Effect on soybean seed germination

Effect of inorganic chemicals on soybean seed germination and radicle growth was determined according to the procedure described earlier (page 35).

Phytotoxicity

This was determined by dipping the roots of soybean seedlings (one month old) in different concentrations of the chemicals prepared in Knop's normal solution. Phytotoxicity was recorded after 48 hours of root dip treatment.

Estimation of total sugars, phenols and amino acids in the chemically treated soybean radicles in vitro

Soybean seedlings were raised from seeds separately in moist chambers made with different concentrations of the inorganic chemicals. When the seedlings were one week old, total sugars, amino acids and phenols of the treated radicles were estimated following Peach and Tracey (1955) for sugar and amino acids and Bray and Thrope's (1954) method for phenols, respectively.

Effect of inorganic amendments on soil microbes

Weighed amount of inorganic fertilisers (0.1, 0.25, 0.5% w/w) were mixed thoroughly with infested soil in

pots (22 cm diameter) and was left for seven days for natural decomposition. The moisture level was maintained at 50% in the pots containing amended soil. Total microbial population and percentage of relative abundance of particular genera/ species of fungi was calculated following the modified soil dilution plate method as described by Baruah and Dutta (1978).

Effect of inorganic amendments on the rhizosphere

Soybean seeds were sown to the soil after one week of amendment and rhizosphere study together with percentage of relative abundance of the particular genera/species of fungi of one month old seedlings, were done following the method described in page 39.

Assessment of disease severity

The disease severity was assessed following the method as described earlier (page 42).

Population dynamics of S. rolfsii in the amended soil

Monthly variation of **S. rolfsii** population in the amended soil was done for a complete cropping season, using slightly modified selective medium as proposed by Backman and Rodriguez-Kabana (1976) as described earlier (page 42).

Effect on plant height

Growth in terms of height of soybean seedlings were measured weekly till they achieved their maximum height.

Dry matter accumulation and yield

Root, shoot, pod weight/plant was calculated and recorded for the seedlings grown in the amended soil. Yield/plant was also determined by calculating the mean weight (gm) of seeds from 30 plants of each treatment/control.

Application of Inorganic fertilizers in the experimental field

Urea (40, 80 kg/ha), $ZnSO_4$ (5, 10 kg/ha) and rock phosphate (40 kg/ha) were applied to naturally infested experimental field soil. After seven days following the application of the amendments, seeds were sown at 5 cm spacing to the plots (5 sq.m) containing eight rows, for planting. The effect of amendments was assessed on the following parameters.

1) Monthly variation of **Sclerotium rolfsii** population in the amended soil.
2) Seedlings/plants height (fortnightly).
3) Disease severity (i.e. percentage of infected plants, assessed fornightly).
4) Yields (q/ha).

RESULTS

Effect of amendments on in vitro growth

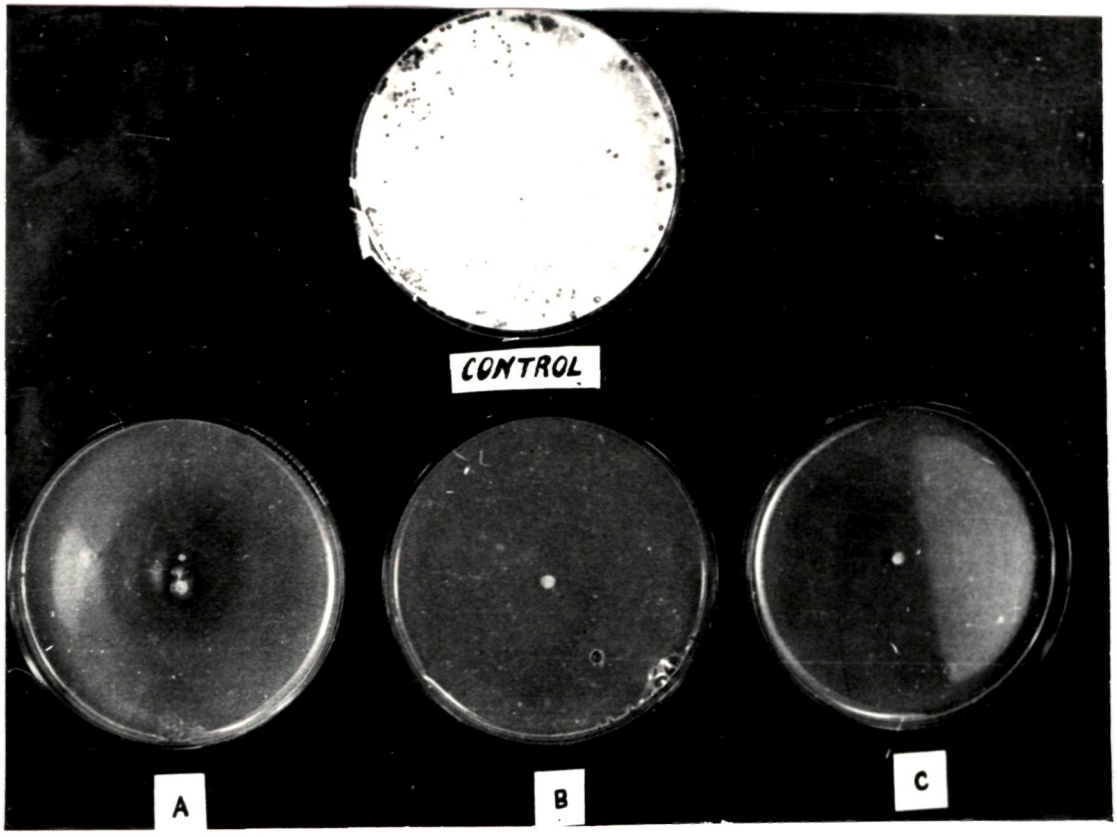
Among the inorganic chemicals used in the present work, urea (0.25%, 0.5% 1% w/v) and $ZnSO_4$ (0.5 and 1% w/v) showed higher inhibitory effect on the radial expansion of **S.rolfsii** in culture (plate 8). Inhibition increased with the increase in the concentration of the chemicals. Comparatively very

Plate 8: Showing the effect of inorganic chemicals on the growth of **Sclerotium rolfsii in vitro.**

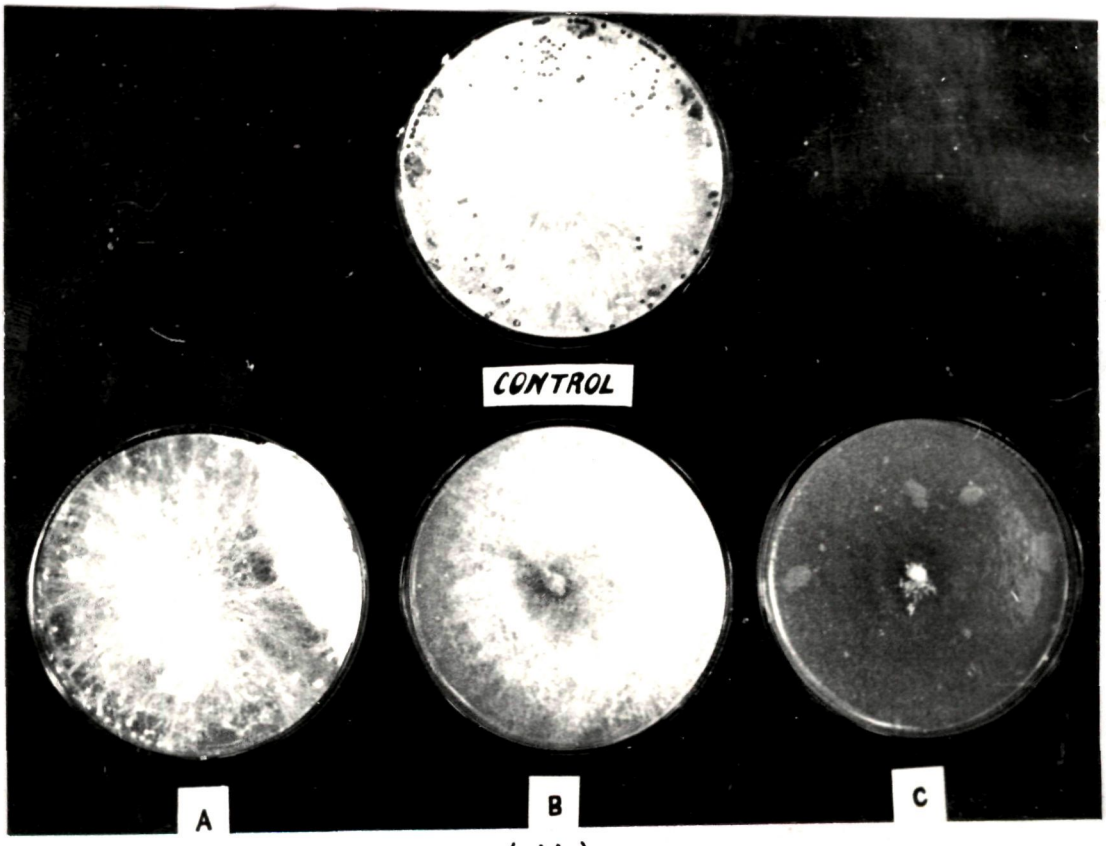
(i) Control (without chemical) and Urea: A=0.25%w/v, B=0.5%w/v, C=1.0%w/v.

(ii) Control (without chemical) and zinc sulphate: A=0.10%w/v, B-0.25%w/v, C=1.0%w/v.

PLATE-8



(i)



(ii)

little inhibitory effect was observed with the rock phosphate and calcium nitrate (Fig. 5.1). An interesting observation was made in case of calcium carbonate, where a reduction in size of the sclerotium was observed.

Effect on the sclerotial germination

Inhibition in sclerotial germination was also recorded as follows: Zinc sulphate (0.25,0.5%w/v), Urea (0.5%w/v) gave 100% inhibition, whereas, ammonium nitrate (0.25,0.5%w/v)and $ZnSO_4$ (0.1%w/v) showed 50% reduction. Higher concentration of almost all the inorganic fertilizers, showed inhibitory effect on the sclerotial germination in aqueous solution(Table 3.1).

Survibality of sclerotium in amended soil

It was observed that the survibality of sclerotium decreased with the increase in concentration of the chemicals as well as with the period of incubation. Apparently, calcium nitrate, urea and ammonium nitrate, although, showed reduced germinability (as observed from the recovered sclerotia) but as a matter of fact the recovery of empty sclerotial shell suggests that they must have been germinated in the soil (as evidenced from the mycelial growth and new sclerotia formation). Therefore, if the empty shells were also taken into consideration, the figure of germinated sclerotia in the laboratory observation will give altogether a confusing picture. Which means that in the initial stage these chemicals boosted the germination ability of the sclerotium and the germinated sclerotial mycelium probably gets lysed in absence of an

Fig. 5.1: Effect of inorganic chemicals (i.e. A. Calcium carbonate, B. Calcium nitrate, C. Rock phosphate, D. Zinc sulphate, E. Urea, F. Ammonium nitrate) on the growth of **Sclerotium rolfsii in vitro.**

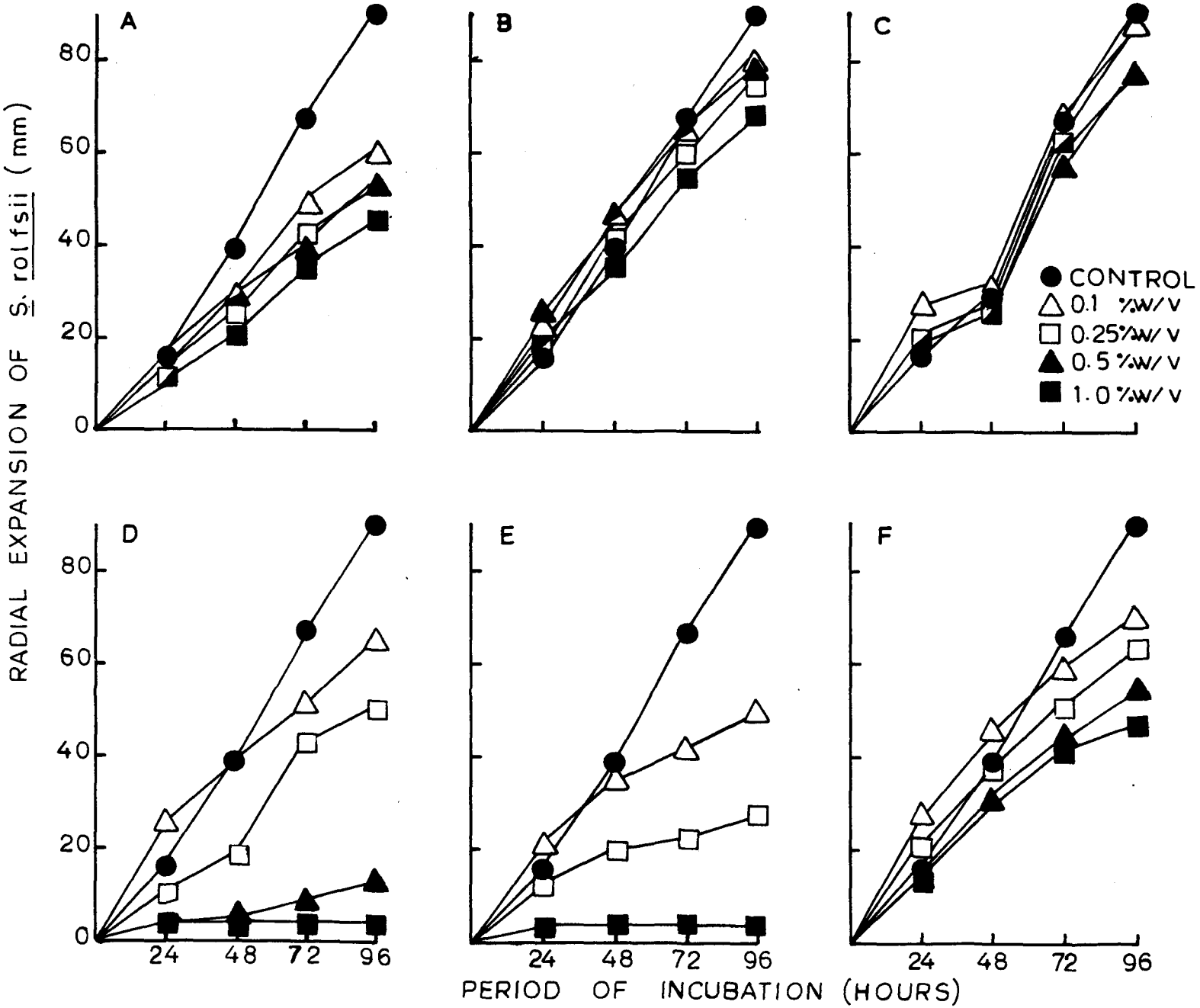

A
B
C
D
E
F
80
60
40
20
0
24
48
72
96
RADIAL EXPANSION OF S. rolfsii (mm)
PERIOD OF INCUBATION (HOURS)
CONTROL
0.1 %W/V
0.25%W/V
0.5 %W/V
1.0 %W/V

FIG. 5·1

TABLE 3.1 : Effect of Inorganic amendments on the survibality of Sclerotium.

Amendments	Concentration (%)	Germination in PDA[1] amended with Inorganic chemicals (%)	Sclerotial germination(%)				
			In aqueous solution after 7 days)[1]	In soil[2] (Period of Incubation(Days))			
				15	30	45	60
Calcium carbonate	0.10	100.00	100.00	66.70	58.30	50.00	37.50
	0.25	80.00	100.00	69.20	47.00	41.70	21.40
	0.50	80.00	65.00	53.30	50.00	46.70	30.70
Calcium nitrate	0.10	100.00	65.00	61.50	36.30	-	-
	0.25	100.00	50.00	60.00	30.00	-	-
	0.50	100.00	35.00	53.30	-	-	-
Rock phosphate	0.10	100.00	100.00	100.00	100.00	93.70	93.30
	0.25	100.00	100.00	80.00	78.60	75.00	60.00
	0.50	100.00	90.00	86.70	73.90	50.00	50.00
Zinc sulphate	0.10	50.00	70.00	66.70	60.00	41.70	23.30
	0.25	-	60.00	53.80	44.40	25.00	10.00
	0.25	-	45.00	50.00	38.90	16.70	-
Urea	0.10	100.00	100.00	69.20	63.60	14.20	-
	0.25	100.00	70.00	58.30	33.30	-	-
	0.50	-	30.00	40.00	33.30	-	-
Ammonium nitrate	0.10	100.00	100.00	66.70	35.70	17.64	1.70
	0.25	50.00	70.00	42.85	36.30	12.50	-
	0.50	50.00	50.00	43.30	38.50	11.10	10.00
Control (without amendment)	-	100.00	85.00	100.00	100.00	100.00	100.00

1) Calculation based on 60 sclerotia in each case.
2) Calculation based on recovered sclerotia.
(-) No germination.

appropriate host. In other fertiliser amended soil due to their toxic effect, the sclerotia were found to loss their viability. Whereas in nonamended control soil 100% sclerotia were found to remain viable (Table 3.1).

Effect on seed germination and radicle growth

Most of the inorganic chemicals used showed varied toxic effects (excepting zinc sulphate) on the germination of seeds and radicle growth. Higher concentration of the chemicals showed inhibitory effect. Increased inhibition in germination was observed with urea and ammonium nitrate treatments (0.25, 0.5% w/v). Reduced radicle growth was observed in case of zinc sulphate and urea treatments (Table 3.2).

Phytotoxicity

Visual observation on the toxic nature of the chemicals viz. zinc sulphate, urea and ammonium nitrate was recorded after 48 hours of root dip treatment. Higher concentrations (0.25, 0.5%w/v) of the chemicals were found to be toxic to the plants, showing flagging and drooping of the leaves (Table 3.2).

Effect on the total sugars, phenols and amino acids

Most of the treated soybean radicles (excepting a few) showed a significant decrease in the total sugars and amino acids, whereas, phenolic content was found to be increased (Table 3.3). Higher concentration of urea and ammonium nitrate

TABLE 3.2 : Effect of chemicals in aqueous solution and soil amendments on the seed germination/radicle growth and pre-emergence rot of soybean seeds.

Inorganic chemical solution/soil amendments	Concentration (%)	Effect of chemicals in aqueous solution **in vitro**			Effect of soil amendments **in vivo**	
		Seed germination (%)[1]	Radicle growth with ±S.E. (Cm)[1]	Phyto-toxicity[2]	Seed germination (%)[3]	Pre-emergence rot (%)[3]
Calcium carbonate	0.10	100.00	13.86±1.58	-	91.70	8.30
	0.25	85.00	8.37±0.80	-	83.30	16.70
	0.50	85.00	7.13±1.12	-	86.60	13.40
Calcium nitrate	0.10	90.00	8.78±1.55	-	91.70	8.30
	0.25	85.00	7.93±1.00	-	85.00	15.00
	0.50	75.00	6.20±0.89	-	76.60	23.40
Rock phosphate	0.10	100.00	16.00±1.36	-	96.60	3.40
	0.25	90.00	9.00±1.23	-	88.30	11.70
	0.50	80.00	6.63±0.86	-	80.00	20.00
Zinc sulphate	0.10	100.00	6.56±0.84	-	88.30	11.70
	0.25	100.00	6.57±0.87	-	81.60	18.30
	0.50	100.00	5.87±0.89	+	76.60	23.40
Urea	0.10	90.00	5.43±0.65	-	91.70	8.30
	0.25	40.00	5.25±0.82	+	65.00	35.00
	0.50	10.00	3.00±0.00	++	63.30	36.70
Ammonium nitrate	0.10	85.00	6.63±1.02	-	83.30	16.70
	0.25	60.00	7.57±1.09	+	75.00	25.00
	0.50	40.00	8.29±1.47	+	70.00	30.00
Control (without treatment)	-	100.00	9.65±1.23	-	86.60	13.40

1) Calculations based on 20 seeds after 7 days Incubation;
2) Observation based on 10 seedlings; (-) non toxic; (+) slightly toxic; (++) toxic.
3) Calculation based on 60 seeds in each case.

TABLE 3.3 : Estimation of total sugars, phenols and amino acids (µg/100 mg dry wt)[1] in soybean radicles grown in the aqueous solution with test chemicals **in vitro.**

Inorganic chemical treatment	Concentration(%)	Total sugars	Total phenols	Total amino acids
Calcium carbonate	0.10	185.00	4.00	140.00
	0.25	190.00	2.50	170.00
	0.50	135.00	4.00	130.00
Calcium nitrate	0.10	180.00	5.00	180.00
	0.25	195.00	8.00	200.00
	0.50	190.00	5.00	200.00
Rock phosphate	0.10	212.00	4.00	160.00
	0.25	185.00	8.00	190.00
	0.50	180.00	9.00	210.00
Zinc sulphate	0.10	190.00	9.00	158.00
	0.25	145.00	6.00	130.00
	0.50	125.00	8.00	80.00
Urea	0.10	225.00	10.00	240.00
	0.25	190.00	8.00	230.00
	0.50	185.00	11.00	236.00
Ammonium nitrate	0.10	175.00	9.00	244.00
	0.25	180.00	8.00	260.00
	0.50	180.00	12.00	266.00
Control (without treatment)	-	225.00	4.00	210.00

1) Mean of three replicates in each case.

accumulated more amino acids and phenolic substances in the radicles compared to other treatments and control.

Effect of inorganic amendments on the soil microorganisms

A considerable increase in the fungal population was observed after the soil treated separately with the chemical fertilizers (i.e. rock phosphate, zinc sulphate and ammonium nitrate) (Table 3.4). Actinomycete and bacteria also increased due to amendments in the infested soil. Comparatively, higher population of actinomycete and bacteria was observed with calcium nitrate at 0.5% and 0.1% (w/w) respectively.

Effect of amendments on particular genera of fungi in soil

Soil amended with $ZnSO_4$ (0.25%) boosted Aspergilli, whereas, Mucorales was found to be stimulated in almost all the treated soil (Table 3.5). Calcium nitrate (0.25%) showed an increase of **Penicillium** spp. over the control. Other genera like **Cephalosporium, Cladosporium, Fusarium, Geotrichum** and **Gliocladium** spp. were also found to be present in the treated soil with an increase in their population (Table 3.5). Altogether twenty seven species belonging to seventeen genera have been isolated from the soil and recorded in Table 3.6. Six species of **Aspergillus**, five species of **Penicillium, Rhizopus stolonifer** and red yeast were found to be dominant in the amended soil.

TABLE 3.4 Effect of Inorganic amendments on the soil micro-organisms.

Amendments	Concentration (%)	Total soil microflora in thousands g^{-1} dry soil[1]		
		Fungi (10^4)	Actinomycete (2×10^4)	Bacteria (3×10^4)
Calcium carbonate	0.10	18.00	246.00	91.70
	0.25	13.00	284.50	111.00
	0.50	10.00	304.00	144.00
Calcium nitrate	0.10	16.20	220.50	271.00
	0.25	14.00	319.00	230.00
	0.50	7.90	358.50	94.70
Rock phosphate	0.10	23.00	188.00	95.00
	0.25	15.80	158.00	101.70
	0.50	15.00	241.50	116.00
Zinc sulphate	0.10	18.00	200.00	118.30
	0.25	18.30	244.00	121.30
	0.50	21.60	165.50	159.00
Urea	0.10	7.00	227.50	76.00
	0.25	8.80	169.00	101.70
	0.50	11.70	157.50	103.00
Ammonium nitrate	0.10	18.70	132.50	130.30
	0.25	18.00	143.00	131.00
	0.50	18.30	202.50	124.00
Infected control (without amendment)	-	10.40	62.50	44.20
Significance by F test (1%)	-	Yes	Yes	Yes

1) Mean of three replicates in each case.

TABLE 3.5 : Effect of inorganic soil amendments on the percentage of relative abundance of particular genera of fungi in pathogen infested soil.

Amendments	Concentration (%)	Aspergilli	Cephalosporium spp.	Cladosporium spp.	Fusarium spp.	Geotrichum spp.	Gliocladium spp.	Mucorales	Penicillia	Trichoderma spp.	Yeast	Other genera of fungi imperfecti
Calcium carbonate	0.10	20.00	7.00	-	-	18.00	-	29.50	9.00	-	11.00	5.00
	0.25	19.00	-	8.33	-	15.00	-	20.00	12.00	-	21.00	5.00
	0.50	14.00	6.30	5.50	-	14.00	-	10.90	27.00	-	10.90	11.40
Calcium nitrate	0.10	3.12	-	-	-	-	-	21.87	24.92	12.50	21.80	15.52
	0.25	5.10	2.56	-	2.56	10.25	-	10.25	33.20	2.56	23.07	10.25
	0.50	10.52	5.26	-	-	21.05	-	15.80	5.26	-	21.05	21.05
Rock phosphate	0.10	15.15	-	-	-	18.18	-	6.06	30.28	3.03	27.30	-
	0.25	11.80	11.76	-	5.88	23.50	-	17.64	2.90	-	26.50	-
	0.50	7.14	21.42	-	-	14.28	-	25.01	-	25.01	-	7.14
Zinc sulphate	0.10	-	34.09	-	-	25.00	-	20.49	4.54	-	11.36	4.54
	0.25	34.77	21.73	-	-	-	-	21.73	13.04	8.69	-	-
	0.50	4.65	20.90	4.65	-	6.97	-	18.55	-	18.57	25.58	-
Urea	0.10	-	29.40	-	-	-	-	17.70	-	23.60	29.40	-
	0.25	8.00	-	8.00	-	16.00	-	16.00	28.00	12.00	12.00	-
	0.50	3.57	10.71	-	17.85	-	-	21.44	17.85	7.14	21.40	-
Ammonium nitrate	0.10	-	11.10	-	4.40	-	20.00	4.44	13.30	17.80	22.20	6.70
	0.25	3.30	6.70	-	-	16.70	-	33.30	6.60	6.70	26.70	-
	0.50	-	-	21.60	-	13.50	-	24.30	-	10.80	24.30	5.40
Control (without amendment)	-	16.70	-	-	-	19.40	-	5.60	27.80	2.80	13.90	13.90

TABLE 3.6 : Effect of Inorganic amendments to soil on the percentage of relative abundance of fungi under infested condition.

Soil fungi	Control (without amendment)	Calcium carbonate			Calcium nitrate			Zinc sulphate			Rock phosphate			Urea			Ammonium nitrate		
		0.10	0.25	0.50	0.10	0.25	0.50	0.10	0.25	0.50	0.10	0.25	0.50	0.10	0.25	0.50	0.10	0.25	0.50
Absidia repens van Tieghem.	-	14.00	-	-	-	-	-	-	-	4.65	-	-	-	-	-	-	-	16.70	-
Acremonium sp.	-	-	-	-	3.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus candidus Link ex. Fries.	-	-	-	-	-¢	-	-	-	-	-	-	-	-	-	-	-	-	3.30	-
A. flavipes Thom. & Church.	8.30	-	-	8.00	-	-	10.52	-	-	-	-	-	7.14	-	-	-	-	-	-
A. flavus Link ex. Fries.	-	-	7.60	6.00	-	5.10	-	-	-	4.65	-	5.90	-	-	8.00	-	-	-	-
A. niger van. Tieghem.	8.40	20.00	-	-	-	-	-	-	8.69	-	15.15	5.90	-	-	-	3.57	-	-	-
A. versicolor (Vuillemin) Tiraboschi.	-	-	11.40	-	3.10	-	-	-	26.08	-	-	-	-	-	-	-	-	-	-
Cephalosporium roseo-griseum Saksena.	-	7.00	-	6.30	-	2.56	5.26	34.09	21.73	20.09	-	11.76	21.42	29.04	-	10.71	11.10	6.70	-
Cladosporium herbarum. Link ex. Fries.	-	-	8.30	5.50	-	-	-	-	-	4.65	-	-	-	-	8.00	-	-	-	21.06
Fusarium oxysporum	-	-	-	-	-	2.56	-	-	-	-	-	5.88	-	-	-	17.85	4.40	-	-
Geotrichum sp.	19.40	18.00	15.00	14.00	-	10.25	21.05	25.00	-	6.97	18.18	23.50	14.28	-	16.00	-	-	16.70	13.50
Gliocladium catenulatum Gilman & Abbott.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20.00	-	-
Helminthosporium sp.	-	-	5.00	4.00	-	-	15.08	-	-	-	-	-	-	-	-	-	6.70	-	-
Humicola sp.	-	4.50	10.00	-	-	-	-	9.00	-	11.60	-	-	3.57	-	-	7.14	-	-	-
Mucor circinelloides Van Tieghem.	2.80	-	-	10.90	-	-	-	-	-	-	-	-	7.14	5.90	-	-	-	-	-
Penicillium brefeldianum Dodge.	5.60	9.00	-	15.00	-	5.10	5.26	-	-	-	-	-	-	-	-	17.85	13.30	3.30	-
P. chrysogenum Thom.	-	-	-	12.00	12.50	5.10	-	-	13.04	-	18.18	2.90	-	-	16.00	-	-	3.30	-
P. oxalicum Thom.	-	-	-	-	3.10	-	-	-	-	-	-	-	-¢	-	-	-	-	-	-
P. rubrum Stoll.	11.10	-	12.00	-	9.30	23.00	-	4.54	-	-	12.10	-	-	-	12.00	-	-	-	-
P. vermiculatum Dangeard.	11.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	¢-
Rhizopus stolonifer (Ehrenb. ex. Link) Lind 1913.	2.80	11.00	10.00	-	21.90	10.25	15.80	11.40	21.73	2.30	6.00	17.64	14.30	11.80	16.00	14.30	4.40	16.60	24.30
Torula sp.	-	-	-	-	9.30	10.25	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichoderma hazianum Rifai.	-	-	-	-	12.50	-	-	-	-	-	-	-	-	11.80	-	-	-	-	-
T. koningii oudemans.	2.80	-	-	-	-	-	-	-	-	11.60	3.03	-	10.70	-	12.00	7.14	11.10	6.70	2.70
T. viride Pers. ex. Fries.	-	-	-	-	-¢	2.56	-	-	8.69	6.97	-	-	14.30	11.80	-	-	6.70	-	8.10
Verticillium sp.	-	-	-	5.40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Yeast (red)	13.90	11.00	21.00	10.90	21.90	23.07	21.05	11.36	-	25.58	27.30	26.50	-	29.40	12.00	21.40	22.20	26.70	24.30
Unidentified	13.90	4.50	-	2.00	3.12	-	5.26	4.54	-	-	-	-	7.14	-	-	-	-	-	5.40

Amendments applied to soil at a rate of 0.10, 0.25, 0.50% w/w.

Effect on the rhizosphere microflora

Almost all the inorganic fertilizers used, decreased the fungal and actinomycete population significantly in the rhizosphere, whereas, bacteria was found to be stimulated. Rock phosphate (0.1, 0.25% w/w) increased the fungal population only, while $ZnSO_4$ and urea (0.5%) increased the bacterial population tremendously in the rhizosphere (Table 3.7).

Effect of amendments on the particular genera of fungi in the rhizosphere

Aspergilli and mucorales were found to be dominant in soybean rhizosphere, irrespective of the amendments used. Rock phosphate (0.25%) and urea (0.5%) boosted Aspergilli while calcium nitrate (0.5%) and ammonium nitrate (0.25%) increased **Fusarium** spp. in the rhizosphere. **Penicillium** spp. were found to be present in all the treatments with comparatively higher population in $ZnSO_4$ (0.1%) treatment. **Trichoderma** spp., **Cephalosporium** sp. and **Geotrichum** sp. were also present in the rhizosphere of soybean in most of the amended soil with a variation in their population (Table 3.8).

Six **Aspergillus**, seven **Penicillium**, four **Trichoderma** and three **Fusarium** species were among the thirtyone species belonging to the fifteen genera of fungi have been isolated from soybean rhizosphere. A white unidentified mycelium also been isolated. **Penicillium chrysogenum, Geotrichum, Rhizopus**

TABLE 3.7 : Effect of Inorganic soil amendments on the rhizosphere microflora of soybean grown in pathogen infested soil.

Soil amendments	Concentration (%)	Rhizosphere micro-organisms in thousands g^{-1} dry soil(1)			Rhizosphere effect (RS/CS)(2)		
		Fungi (10^4)	Actinomycete ($2x10^4$)	Bacteria ($3x10^4$)	Fungi	Actinomycete	Bacteria
Calcium carbonate	0.10	24.00	309.00	304.00	1.20	0.92	1.33
	0.25	19.00	263.00	367.00	0.95	0.79	1.60
	0.50	12.00	253.00	400.00	0.60	0.76	1.75
Calcium nitrate	0.10	28.00	177.00	406.00	1.40	0.53	1.77
	0.25	20.00	319.00	309.00	1.00	0.95	1.35
	0.50	18.00	224.00	321.00	0.90	0.67	1.40
Rock phosphate	0.10	48.00	356.00	389.00	2.40	1.06	1.70
	0.25	40.00	219.00	378.00	2.00	0.65	1.65
	0.50	31.20	209.00	243.00	1.56	0.62	1.06
Zinc sulphate	0.10	12.70	205.00	176.00	0.64	0.61	0.77
	0.25	21.40	216.00	413.00	1.07	0.64	1.80
	0.50	26.90	246.00	431.00	1.35	0.73	1.88
Urea	0.10	24.00	319.00	351.00	1.20	0.95	1.53
	0.25	16.60	224.00	371.00	0.23	0.69	1.62
	0.50	10.00	211.00	432.00	0.50	0.63	1.89
Ammonium nitrate	0.10	10.00	339.00	275.00	0.50	1.01	1.20
	0.25	6.00	210.00	289.00	0.30	0.63	1.26
	0.50	7.40	184.00	300.00	0.37	0.55	1.31
Control rhizospere soil	-	36.59	400.00	270.80	1.82	1.19	1.18
Control soil	-	20.00	335.00	228.75	-	-	-
Significance by F test (1%)	-	Yes	Yes	Yes			

1) Mean of three replicates in each case.

2) Rhizosphere soil/control soil.

TABLE 3.8 : Effect of Inorganic soil amendments on the percentage of relative abundance of rhizosphere fungi of soybean grown in Pathogen infested soil.

Amendments	Concentration (%)	Aspergilli	Cephalosporium spp.	Cladosporium spp.	Fusarium spp.	Geotrichum spp.	Gliocladium spp.	Mucorales	Penicillia	Trichoderma spp.	Yeast	Other genera of fungi imperfecti
Calcium carbonate	0.10	10.86	10.86	-	-	4.34	-	10.86	26.06	4.34	21.70	10.86
	0.25	7.89	13.15	-	2.60	13.15	-	21.10	13.20	10.50	18.42	-
	0.50	6.89	13.78	-	-	10.30	-	10.30	44.76	3.44	6.89	3.44
Calcium nitrate	0.10	8.69	-	-	8.69	8.69	-	17.38	8.69	8.69	39.13	-
	0.25	2.94	14.17	-	11.68	-	8.82	11.76	8.82	5.88	29.40	5.88
	0.50	10.70	14.28	-	14.28	10.70	3.57	7.14	10.70	3.57	17.85	7.14
Rock phosphate	0.10	4.16	-	-	8.30	25.00	-	12.50	8.30	12.50	16.70	12.50
	0.25	27.54	10.30	-	10.30	17.24	-	6.89	-	6.89	20.68	-
	0.50	16.60	-	22.20	-	-	-	11.20	25.00	5.60	19.40	-
Zinc sulphate	0.10	7.14	-	-	-	3.57	-	10.70	67.84	-	-	10.70
	0.25	7.14	7.14	-	3.57	10.70	-	28.55	35.70	3.57	-	3.57
	0.50	6.70	3.30	3.30	30.10	13.30	-	13.40	16.60	-	6.70	6.70
Urea	0.10	4.54	-	-	9.09	13.63	-	13.63	9.09	9.09	27.30	13.63
	0.25	15.38	-	-	7.69	-	-	23.06	34.57	-	19.23	-
	0.50	38.90	-	-	-	-	-	44.50	16.70	-	-	-
Ammonium nitrate	0.10	13.15	7.89	-	13.15	10.52	-	18.41	34.19	2.63	-	-
	0.25	10.70	10.70	-	17.85	28.57	-	14.30	14.28	-	-	3.57
	0.50	8.57	-	-	8.57	22.85	-	31.40	8.60	5.70	14.28	-
Control Rhizosphere soil	-	6.25	-	-	12.50	-	-	3.12	49.92	-	18.72	9.37
Control soil	-	10.40	-	-	2.08	20.83	-	39.73	10.40	-	16.70	-

stolonifer and red yeast were found to be the dominant species in the soybean rhizosphere (Table 3.9).

Assessment on disease severity

Nitrogenous fertiliser amendment to soil initially stimulated the sclerotial germination, resulting pre-emergence rot of soybean seeds. Increased seed rot was observed in the urea (0.25, 0.5%) and ammonium nitrate (0.25, 0.5%) amended soil. Zinc sulphate and calcium nitrate (0.5%) amendment also increased the rot considerably (Table 3.2), compared to non amended infected control. Urea and ammonium nitrate in general was found to break the dormancy (i.e. imposed by soil fungistasis) of the sclerotia resulting an increase in pre/post emergence rot of soybean seeds in soil. Higher concentration of these inorganic fertilizers were also found to be toxic to soybean seed germination and seedling growth.

Although, the above inorganic fertilizers increased pre-emergence rot but the germinated seedlings which escaped rot, showed delayed symptom expression (approx. by two weeks time), in particular with higher concentration of the amendments (i.e. 0.5% w/w) compared to nonamended infected control (Fig. 5.2). Urea (0.25%) and ammonium nitrate (0.5% w/w) amendments found comparatively less effective in reducing the disease severity (Fig. 5.2 E,F), whereas, calcium carbonate, calcium nitrate (0.1% w/w) and rockphosphate at higher concentration (0.5w/w)

TABLE 3.9 : Effect of inorganic amendments to soil on the percentage of relative abundance of rhizosphere fungi under infested condition.

Soil and rhizosphere fungi	Control (without amendment)	Control rhizosphere (without amendment)	Calcium carbonate			Calcium nitrate			Zinc sulphate			Rock phosphate			Urea			Ammonium nitrate		
			0.10	0.25	0.50	0.10	0.25	0.50	0.10	0.25	0.50	0.10	0.25	0.50	0.10	0.25	0.50	0.10	0.25	0.50
Absidia sp.	-	-	-	-	-	4.34	-	-	-	-	-	-	-	5.60	-	-	5.60	-	-	-
Aspergillus candidus Link ex. Fries.	-	-	10.86	2.60	6.89	8.69	-	-	-	-	6.70	-	10.30	-	4.54	-	-	-	-	8.57
A. clavatus Desm.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13.15	-	-
A. flavipes Thom & Church.		6.25	-	-	-	-	-	-	-	-	-	-	-	8.30	-	-	-	-	-	-
A. flavus Link ex.Fries.	10.40	-	-	-	-	-	-	-	-	7.14	-	-	17.24	-	-	-	11.10	-	-	-
A. niger Van Tieghem.	-	-	-	-	-	-	-	-	7.14	-	-	-	-	8.30	-	15.38	-	-	-	-
A. versicolor (Vuillemin) Tiraboschi.	-	-	-	5.30	-	-	2.94	10.70	-	-	-	4.16	-	-	-	-	27.80	-	10.70	-
Cephalosporium acremonium Corda.	-	-	10.86	13.15	13.78	-	14.70	14.28	-	7.14	3.30	-	10.30	-	-	-	-	7.89	10.70	-
Cladosporium herbarum Link ex. Fries.	-	-	-	-	-	-	-	-	-	-	3.30	-	-	22.20	-	-	-	-	-	-
Fusarium oxysporum	2.08	12.50	-	-	-	8.69	5.88	-	-	3.57	13.40	8.30	10.30	-	9.09	-	-	13.15	-	-
F. solani	-	-	-	-	-	-	5.88	7.14	-	-	10.00	-	-	-	-	7.69	-	-	17.85	8.57
Fusarium sp.	-	-	-	2.63	-	-	-	7.14	-	-	6.70	-	-	-	-	-	-	-	-	-
Geotrichum sp.	20.83	-	4.34	13.15	10.30	8.69	-	10.70	3.57	10.70	13.30	25.00	17.24	-	13.63	-	-	10.52	28.57	22.85
Gliocladium catenulatum Gilman & Abbott.	-	-	-	-	-	-	8.22	3.57	-	-	-	-	-	-	-	-	-	-	-	-
Humicola grisea Traaen.	20.83	-	-	5.30	-	-	-	-	-	10.70	-	-	-	-	-	-	27.80	7.89	-	25.70
Mucor hiemalis Wehmer.	18.90	-	-	5.30	-	-	-	-	-	-	6.70	-	-	-	-	11.53	-	-	10.70	-
Penicillium brefeldianum Dodge.	-	3.12	15.20	7.90	-	-	-	10.70	-	-	3.30	-	-	-	-	-	-	-	-	5.70
P. chrysogenum Thom.	10.40	6.24	10.86	5.30	6.90	-	8.82	-	17.90	17.85	10.00	8.30	-	22.20	9.09	-	16.70	7.89	14.28	-
P. granulatum Bainier.	-	3.12	-	-	10.30	-	-	-	10.70	17.85	-	-	-	-	-	15.38	-	13.15	-	-
P. nigricans Bainier.	-	-	-	-	-	-	-	-	7.14	-	-	-	-	-	-	-	-	-	-	2.90
P. oxalicum Thom.	-	-	-	-	13.78	-	-	-	21.40	-	-	-	-	-	-	7.69	-	-	-	-
P. rubrum Stoll.	-	18.72	-	-	-	8.69	-	-	10.70	-	3.30	-	-	2.80	-	-	-	13.15	-	-
P. vermiculatum Dangeard.	-	18.72	-	-	13.78	-	-	-	-	-	-	-	-	-	-	11.50	-	-	-	-
Rhizopus stolonifer (Ehrenb ex. Link)Lind 1913	-	3.12	10.86	10.50	10.30	13.04	11.76	7.14	10.70	17.85	6.70	12.50	6.89	5.60	13.63	11.53	11.10	10.52	3.57	5.70
Torula sp.	-	-	-	-	-	-	-	7.14	-	-	-	12.50	-	-	-	-	-	-	3.57	-
Trichoderma aureoviride Rifai.	-	-	-	7.90	-	8.69	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. harzianum Rifai.	-	-	4.34	2.60	-	-	2.94	-	-	-	-	-	-	-	-	-	-	-	-	-
T. koningii oudemans.	-	-	-	-	3.44	-	2.94	-	-	3.57	-	12.50	6.89	5.60	4.54	-	-	-	-	-
T. viride Pers. ex. Fries.	-	-	-	-	-	-	-	3.57	-	-	-	-	-	-	4.54	-	-	2.63	-	5.70
Verticillium sp.	-	-	10.86	-	-	-	-	-	-	3.57	-	-	-	-	9.09	-	-	-	-	-
Yeast (red)	16.70	18.72	21.70	18.42	6.89	39.13	29.40	17.85	-	-	6.70	16.70	20.68	19.40	27.30	19.23	-	-	-	14.28
Unidentified	-	9.37	-	-	3.44	-	5.88	-	10.70	-	6.70	-	-	-	4.54	-	-	-	-	-

Amendments applied to soil at a rate of 0.10, 0.25, 0.50% w/w.

Fig. 5.2: Effect of inorganic soil amendments (i.e. A. Calcium carbonate, B. Calcium nitrate, C. Rock phosphate, D. Zinc sulphate) on the disease development of soybean caused by **Sclerotium rolfsii.**

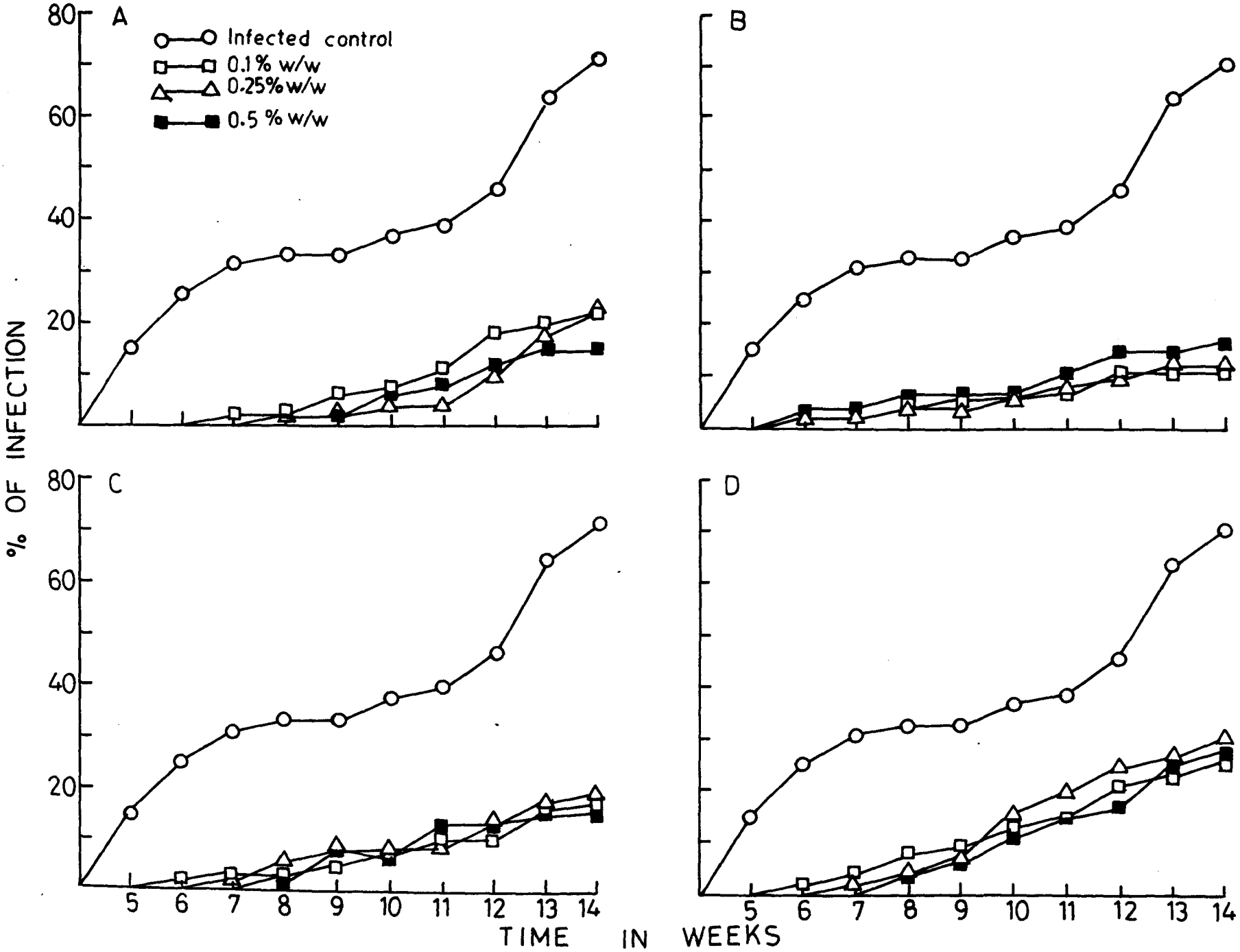
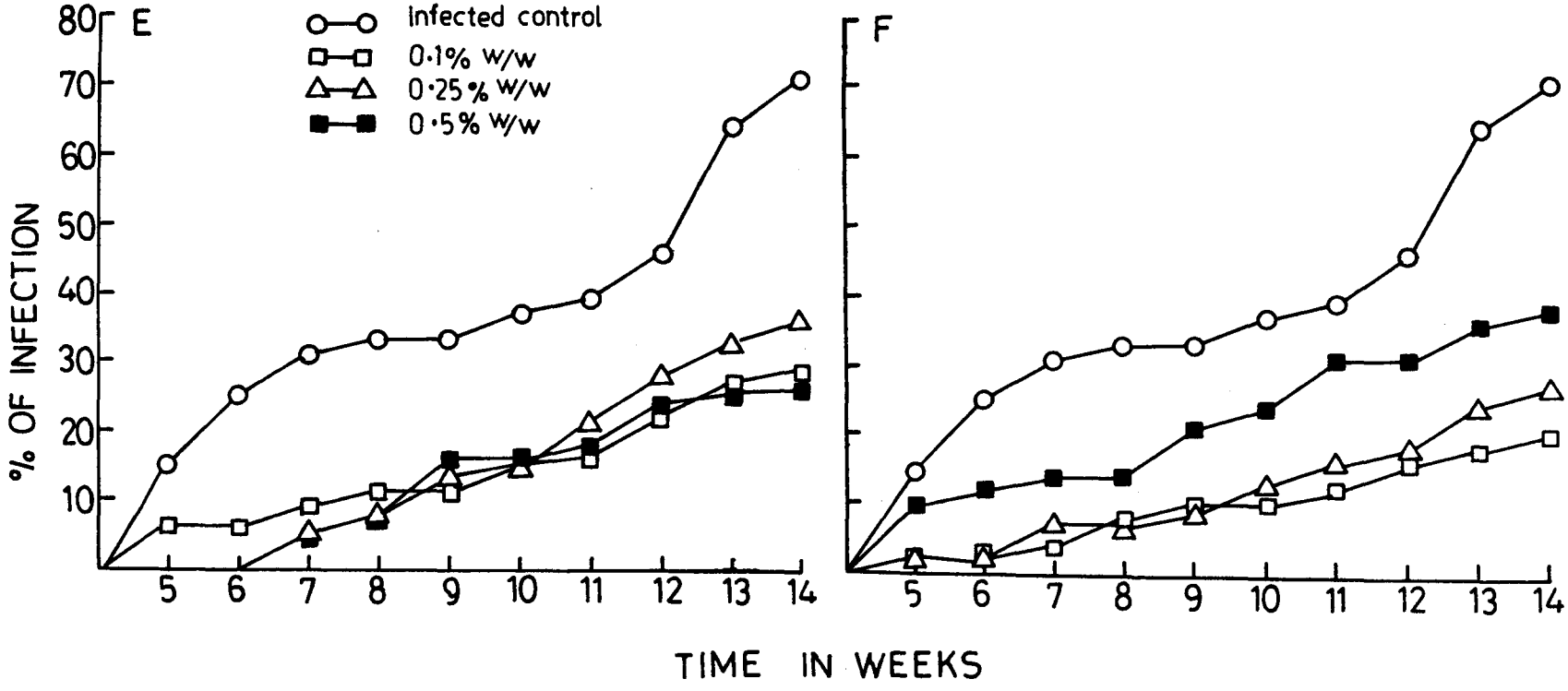

A
B
C
D
Infected control
0.1% w/w
0.25% w/w
0.5 % w/w
% OF INFECTION
80
60
40
20
5
6
7
8
9
10
11
12
13
14
TIME IN WEEKS

Fig.5·2

Fig. 5.2: Effect of inorganic soil amendments (i.e. E. Urea and F. Ammonium nitrate) on the disease development of soybean caused by **Sclerotium rolfsii.**


E
F
Infected control
0·1% W/W
0·25% W/W
0·5% W/W
% OF INFECTION
80
70
60
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9
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13
14
TIME IN WEEKS

Fig. 5·2

reduced the severity of disease to a great extent (Fig. 5.2 A,B,C) (Plate 9). But in all the cases at least 50% control of the disease was achieved.

Population dynamics of Sclerotium rolfsii in the amended soil

An initial increase in **S. rolfsii** population due to fertilizer amendment was observed in all the cases. Maximum increase in population was recorded with urea (0.25, 0.5% w/w) and $ZnSO_4$ (0.1, 0.25% w/w) amendment to soil (Fig. 5.3E,D). This exponential increase in the population of **S. rolfsii** is probably due to the breaking of exogenous dormancy (due to fungistasis) imposed on resting structures i.e. Sclerotium, in the soil. The population declined gradually resulting in the decrease of the disease initiation/severity. In non amended control, the population gradually increased as observed for a complete cropping season.

Effect on the plant height

Soil amendments in most of the cases (excepting a few) reduced the seedling height compared to non amended control (Table 3.10). This reduction seemed to be due to the phytotoxic affect of the inorganic fertilizers used. It was further observed that the increased concentration of the amendments had an adverse affect on the seedlings/plants growth. However, some of the amendments i.e. calcium nitrate (0.1, 0.25% w/w), rock phosphate (0.1, 0.25% w/w) and urea (0.1 w/w)

Plate 9: Showing the reduction in disease severity and increased plant vigour following inorganic soil amendments, viz. (i) calcium nitrate (ii) rock phosphate compared to control (infected). (Control, 0.1, 0.25, 0.5% w/w from left to right).

PLATE-9

(i)

(ii)

Fig.5.3: Effect of inorganic soil amendments (i.e. A. Calcium carbonate, B. Calcium nitrate, C. Rock phosphate, D. Zinc sulphate, E. Urea and F. Ammonium nitrate on the population dynamics of **Sclerotium rolfsii** in natural soil (vertical bar in the figure represent LSD at P=0.05 level).

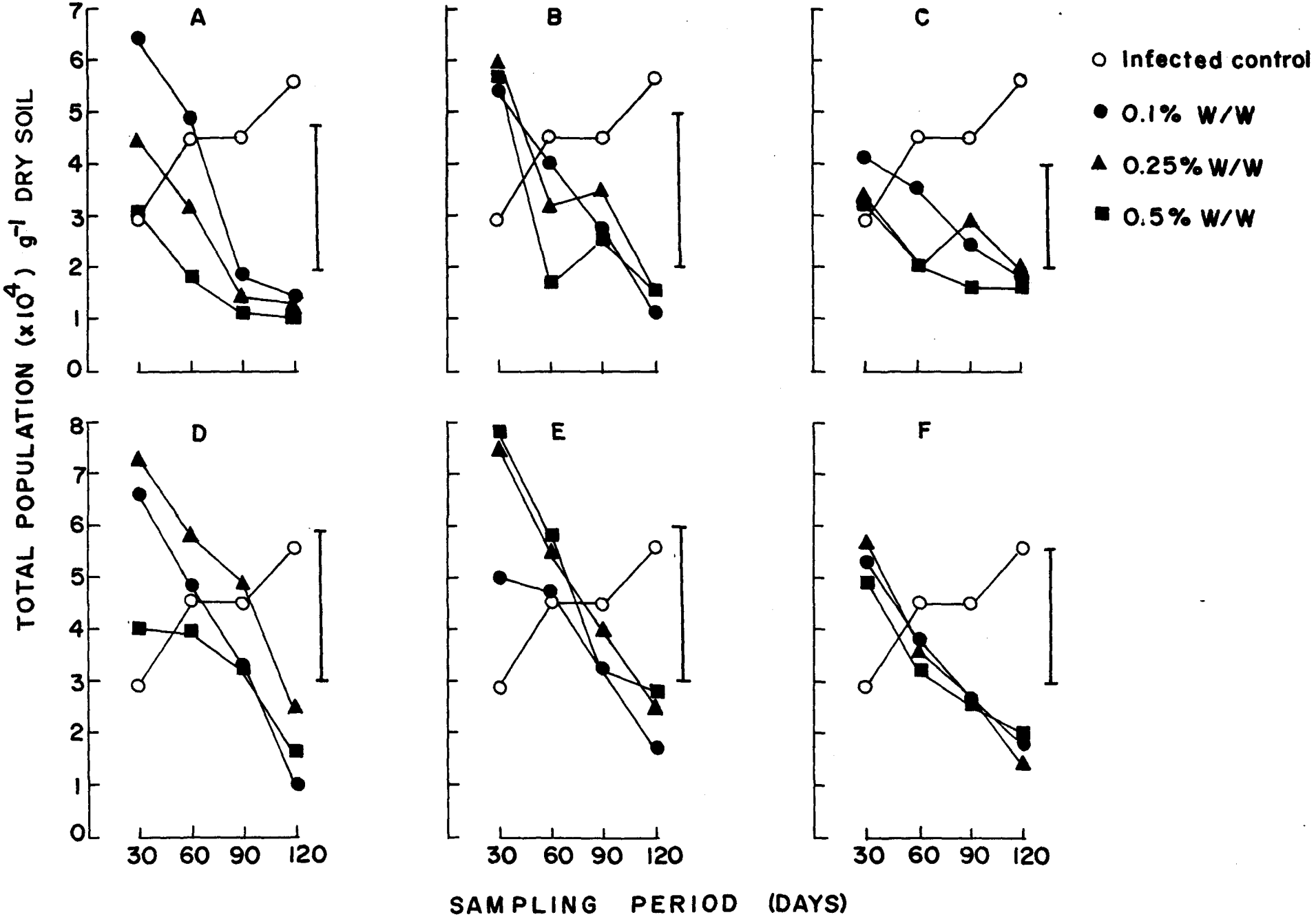

A
B
C
D
E
F
Infected control
0.1% W/W
0.25% W/W
0.5% W/W
TOTAL POPULATION (x10⁴) g⁻¹ DRY SOIL
SAMPLING PERIOD (DAYS)
30 60 90 120

Fig.5·3

TABLE 3.10: Effect of Inorganic soil amendments on the height of soybean plants.(Mean ±S.E.)

Amendments	Concentration (%)	Time in weeks									
		1	2	3	4	5	6	7	8	9	10
Calcium carbonate	0.10	9.70 ±0.52	13.00 ±0.83	15.04 ±0.33	19.70 ±0.61	23.70 ±1.14	27.70 ±0.67	35.75 ±0.96	37.10 ±1.80	46.36 ±2.30	47.22 ±1.37
	0.25	10.20 ±0.96	11.54 ±1.20	14.79 ±0.31	18.28 ±0.45	28.60 ±0.93	28.70 ±0.56	37.56 ±1.22	42.00 ±0.99	47.67 ±1.22	50.00 ±1.66
	0.50	10.50 ±0.67	13.00 ±0.89	15.35 ±0.30	19.40 ±0.68	27.50 ±0.87	29.30 ±0.80	37.81 ±1.19	41.36 ±1.22	47.63 ±2.00	51.80 ±2.31
Calcium nitrate	0.10	9.35 ±0.98	11.60 ±1.10	13.64 ±0.29	18.87 ±0.67	28.54 ±1.36	29.27 ±0.73	35.25 ±1.46	41.27 ±1.43	47.25 ±1.68	50.33 ±1.38
	0.25	10.58 ±0.87	12.00 ±1.03	14.85 ±0.86	18.90 ±0.53	28.90 ±1.21	31.00 ±0.82	36.87 ±1.04	42.50 ±1.19	47.63 ±1.15	51.72 ±1.23
	0.50	10.48 ±0.83	12.10 ±0.98	14.79 ±0.24	19.75 ±0.49	28.33 ±1.45	28.90 ±0.53	37.27 ±1.73	40.85 ±1.22	44.67 ±1.39	48.72 ±1.85
Rock phosphate	0.10	10.98 ±0.99	13.00 ±0.78	16.17 ±0.32	20.15 ±0.42	28.08 ±1.01	31.10 ±0.67	36.56 ±0.78	45.18 ±1.20	49.55 ±1.64	51.10 ±1.48
	0.25	11.25 ±0.89	14.50 ±0.97	15.47 ±0.30	20.25 ±0.52	30.15 ±1.02	32.60 ±0.99	36.50 ±0.84	43.61 ±0.98	49.40 ±1.12	52.40 ±1.10
	0.50	12.90 ±1.20	15.00 ±1.45	16.65 ±0.28	22.30 ±0.45	31.80 ±1.16	35.45 ±1.25	37.67 ±1.13	47.25 ±1.41	48.25 ±1.93	48.30 ±1.53
Zinc sulphate	0.10	9.78 ±1.03	11.00 ±1.60	14.10 ±0.46	18.77 ±0.57	28.70 ±1.40	31.00 ±1.03	37.00 ±0.82	44.90 ±1.55	46.90 ±1.34	49.56 ±2.10
	0.25	9.54 ±0.98	11.30 ±0.90	13.90 ±0.41	18.21 ±0.49	27.00 ±0.79	29.60 ±2.02	31.60 ±1.55	38.62 ±1.89	41.70 ±2.03	47.30 ±1.88
	0.50	8.75 ±1.13	10.15 ±0.89	12.48 ±0.41	16.80 ±0.48	24.40 ±0.79	25.70 ±0.47	29.36 ±0.99	33.30 ±1.59	38.10 ±1.50	39.40 ±1.09
Urea	0.10	9.25 ±0.90	11.38 ±0.56	14.35 ±0.27	18.75 ±0.60	28.50 ±1.06	29.45 ±0.68	35.10 ±0.77	45.10 ±1.44	48.50 ±0.79	54.55 ±1.15
	0.25	10.50 ±0.78	12.00 ±0.67	15.12 ±0.31	17.60 ±0.26	23.68 ±0.99	26.36 ±0.74	32.44 ±0.71	43.00 ±1.02	43.50 ±1.29	46.11 ±2.15
	0.50	8.50 ±0.81	9.20 ±0.82	12.28 ±0.34	13.70 ±0.56	17.40 ±1.02	20.00 ±0.84	26.09 ±1.67	32.72 ±1.83	36.56 ±1.60	38.38 ±1.32
Ammonium nitrate	0.10	10.90 ±0.69	13.00 ±0.97	15.37 ±0.42	20.60 ±0.63	30.60 ±0.75	30.40 ±0.88	36.18 ±0.81	44.09 ±2.47	48.45 ±1.53	51.00 ±2.15
	0.25	9.75 ±0.97	11.00 ±0.72	13.37 ±0.36	15.75 ±0.81	24.18 ±1.56	26.40 ±1.53	31.88 ±2.34	41.70 ±2.17	48.00 ±3.13	48.00 ±4.38
	0.50	7.70 ±1.40	9.00 ±0.47	11.69 ±0.41	14.20 ±0.73	19.20 ±1.24	20.00 ±1.21	29.40 ±1.58	35.20 ±2.48	36.97 ±0.65	39.44 ±1.42
Infected control (without amendment)	-	7.50 ±0.88	11.35 ±0.76	15.19 ±0.40	17.10 ±0.76	22.07 ±1.35	24.06 ±0.95	37.90 ±1.70	40.70 ±2.28	47.50 ±2.04	51.00 ±2.79

amended to soil increased plant vigour compared to other inorganic amendments (Plate 9 & 10).

Effect of inorganic soil amendments on dry matter accumulation in soybean plant.

Shoot weight/plant increased with most of the treatments. Urea (0.1%w/w), calcium nitrate (0.25% w/w) and calcium carbonate (0.1, 0.25% w/w) increased the dry matter accumulation of the above ground parts of the seedlings (7.0, 6.5, and 6.18g/ plant respectively). Whereas, higher concentration of urea (0.25, 0.5% w/w) and ammonium nitrate (0.5% w/w) decreased the same (Table 3.11).

Increased dry weight of root (Table 3.11) was also observed with urea (all the concentration) and zinc sulphate (0.1% w/w). But reduction in leaf area and number of leaves was visually observed in the plants grown in amended soil.

Increased pod weight/plant was also observed in most of the cases excepting in higher concentration (i.e. 0.25, 0.5% w/w) of urea and ammonium nitrate (Table 3.11). Higher pod weight was recorded after rock phosphate and zinc sulphate (in all the concentrations used) amendments.

Effect on the soybean yield

Yield (gm) obtained from plants grown in amended soil was found to be non-satisfactory (Table 3.11). Comparatively,

Plate 10: Showing soybean plants grown in urea amended soil

(i) In pots: Infected control, urea amended soil i.e. 0.1, 0.25, 0.5%w/w (left to right);

(ii) Under field condition: Increased seedling rot following urea (80kg/ha) amendment to soil.

PLATE-10

(i)

(ii)

TABLE 3.11 : Effect of Inorganic soil amendments on the dry matter accumulation and yield of soybean plants.[1]

Amendments	Concentration(%)	Dry weight of shoot/plant (gm)	Dry weight of Root /Plant (gm)	Pod weight/ Plant (gm)	Yield/ Plant (gm)
Calcium carbonate	0.10	6.18	2.10	5.59	4.09
	0.25	6.18	1.99	5.60	4.36
	0.50	6.00	1.75	5.37	4.20
Calcium nitrate	0.10	5.50	2.25	5.50	4.30
	0.25	6.50	2.30	5.67	4.00
	0.50	5.00	2.30	5.20	3.64
Rock phosphate	0.10	5.50	2.45	6.50	4.50
	0.25	5.00	2.40	7.00	4.67
	0.50	6.00	2.42	6.00	4.00
Zinc sulphate	0.10	5.31	2.85	6.65	4.15
	0.25	5.00	2.20	6.50	4.25
	0.50	4.80	1.80	6.00	4.00
Urea	0.10	7.00	3.00	6.50	5.00
	0.25	4.92	2.82	5.00	4.15
	0.50	4.33	2.70	4.50	3.90
Ammonium nitrate	0.10	5.50	2.10	5.00	3.40
	0.25	6.00	1.99	4.70	3.00
	0.50	4.25	1.80	4.50	3.00
Infected control (without amendment)	-	5.00	2.50	5.00	4.00
LSD(P=0.05)	-	1.47	0.41	0.85	0.42

1) Mean of 30 plants in each case.

poor yield that produced by the plant, seems to be due to the toxic effect of the inorganic chemicals, and their concentrations used, which directly affected the plant height and yield, suggests to reduce the doses of fertilizers used.

Result of the experiments under field condition

(i) **Assessment of S. rolfsii population in the experimental soil**

The population of **S. rolfsii** was found to have increased after inorganic fertilizer amendment to soil. In some cases, thin mycelial mats have been observed following the application of the amendments due to the rapid growth and multiplication of sclerotium in the soil. Urea (40 and 80kg/ha) amendment, increased the population compared to non amended control. Higher concentration of urea and $ZnSO_4$ showed an initial increase in the population, which subsequently reduced slightly with time as observed in the subsequent samplings (Fig. 5.4). Whereas, rock phosphate amendment (40 Kg/ha) showed a gradual increase in the **S. rolfsii** population, which is similar to non amended control (Fig. 5.4). Breaking up of dormancy of sclerotium due to inorganic amendments resulted an increase in the population at the initial stage (i.e. after 30 days) but with time it declined, probably due to the autolysis and/or due to the absence of sufficient nutrients required for multiplication and formation of resting structures.

Fig. 5.4: Effect of inorganic soil amendments on the population dynamics of **Sclerotium rolfsii** under field condition (vertical bar in the figure represent LSD at P=0.05 level).

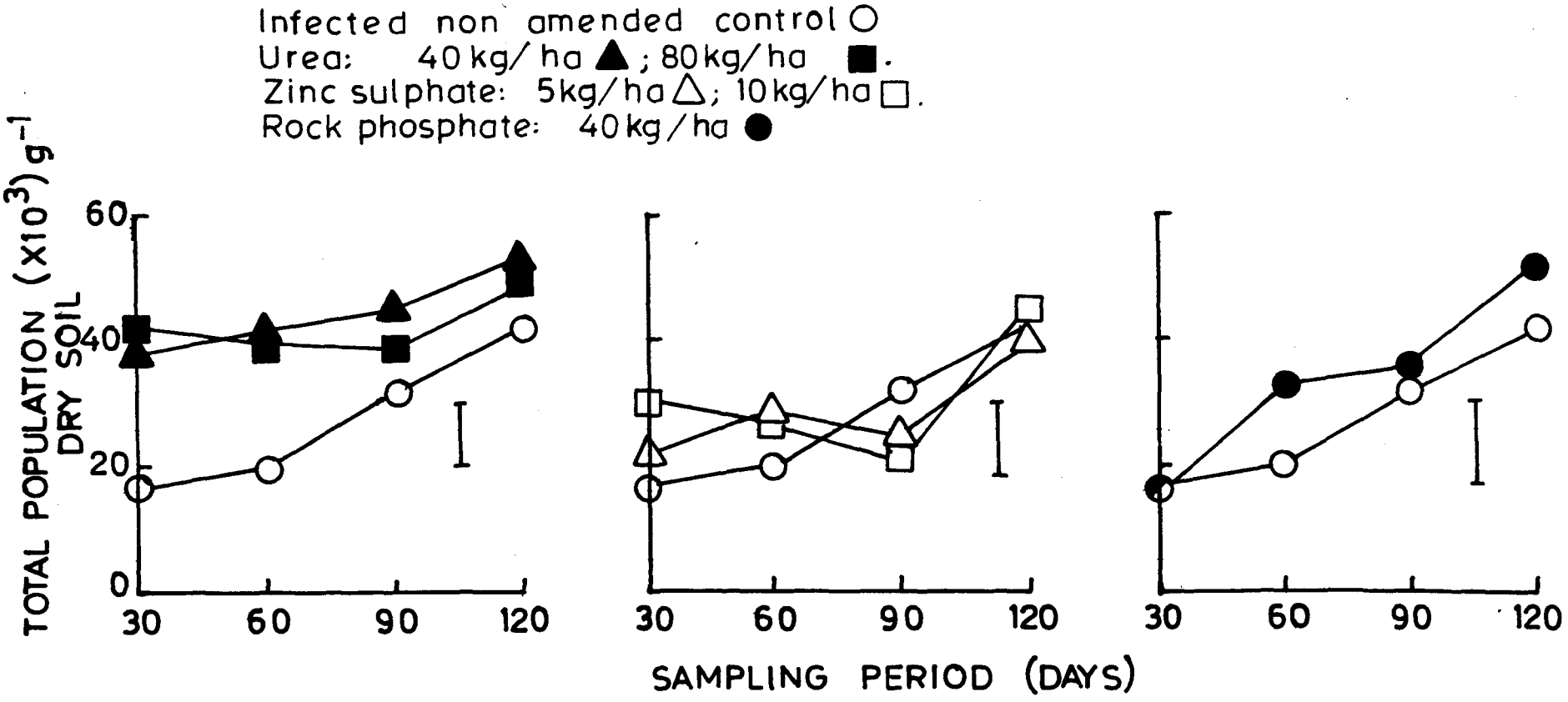

Infected non amended control ○
Urea: 40 kg/ ha ▲ ; 80 kg/ ha ■.
Zinc sulphate: 5 kg/ ha △ ; 10 kg/ ha □.
Rock phosphate: 40 kg / ha ●
TOTAL POPULATION (X10^3) g^{-1} DRY SOIL
60
40
20
0
30
60
90
120
SAMPLING PERIOD (DAYS)

FIG.5·4

(ii) **Disease severity**

The seedlings grown in the urea amended soil (80 kg/ha) showed high rate of infection within 15 days after seed germination (Plate 10). In case of other amendments, the rate and progress of disease development was found to be very slow, compared to non amended infected control. Infection rate increased gradually and was found to reach its peak, when the seedlings were ten weeks old. In general, almost in all the cases, a reduction in disease severity was recorded (Table 3.12). Maximum reduction in the seedlings/plants infection was obtained with $ZnSO_4$ amendment to soil.

(iii) **Effect on plant height**

Urea (80 kg/ha) and $ZnSO_4$ (10 kg/ha) amendment to soil, stimulated the growth (i.e. height) and vigour of the plants (Table 3.12), increased number of branches and leaf area (visual observations), compared to the non amended infected control. Lower concentration of these fertilizers found to have very little effect on plant height and vigour (Plate 11).

(iv) **Effect on yield**

The fertilizers used in the present work invariably increased soybean yield. Higher increase was observed with urea (80 kg/ha) followed by $ZnSO_4$ (10 kg/ha). Other concentrations of the inorganic fertilizers used were also produced

TABLE 3.12 : Effect of Inorganic soil amendments on the height[1] of soybean plants under field condition (values in Parenthesis indicate percentage of infected plants[2]).

Amendments	Concentration (Kg/ha)	Time in weeks				
		2	4	6	8	10
Urea	40	14.90±0.27 (8.75)	26.00±1.06 (17.86)	53.00±0.76 (22.20)	72.70±1.45 (30.30)	90.74±1.70 (43.93)
	80	18.59±0.71 (20.50)	28.40±0.93 (22.70)	58.00±0.80 (34.88)	90.50±1.73 (38.20)	104.75±1.45 (39.13)
Zinc sulphate	5	11.50±0.41 (9.50)	20.40±0.42 (15.56)	50.90±0.61 (21.95)	68.67±1.14 (28.50)	82.41±1.77 (35.70)
	10	16.80±0.25 (8.62)	24.60±0.59 (12.12)	55.70±0.84 (19.44)	87.50±4.95 (30.90)	101.00±1.20 (34.00)
Rock phosphate	40	14.00±0.46 (6.30)	23.42±0.65 (19.70)	52.92±2.39 (35.20)	73.00±4.10 (37.90)	86.66±4.95 (41.30)
Infected control (Non-amended)	-	11.00±0.41 (22.00)	18.10±1.01 (36.58)	52.95±1.25 (43.30)	75.20±4.34 (54.00)	87.04±1.45 (65.85)

1) Mean of 10 plants with ±S.E.
2) Calculations based on 100 plants (approx.)/plot (5 sq.m) in each case.

Plate 11: Showing the reduction in disease severity in soybean plants grown in infested field soil, following soil amendments i.e. (i) Urea, (ii) Rock phosphate.

PLATE-II

(i)

(ii)

a comparatively better yield over non amended infected control (Fig. 5.5).

DISCUSSION

Inorganic amendments are known to have effects on plants either direct, i.e. without mediation of soil micro-organisms, or indirect, through biological transformation. They may change host structure, composition and metabolism and so affect the host as a medium for the growth and reproduction of the pathogen. Most of the inorganic soil amendments used in the present study, suppressed the mycelial growth and sclerotial germination of **S. rolfsii in vitro**. Urea and $ZnSO_4$ produced comparatively higher inhibition (95.5%) on the radial growth and sclerotial germination, suggesting their fungitoxic nature. Several workers suggested that the inorganic fertilizers (i.e. ammonium fertilizer) may directly inhibit the sclerotial germination and retard the mycelial growth of **S. rolfsii**, specially if ammonia is released by them (Henis and Chet, 1967; Avizohar-Hershenzen and Shacked, 1969; Punja and Grogan, 1982; Punja **et al**, 1985) or by changing the host susceptibility or indirectly by increasing the population of the antagonistic soil microorganisms (Henis and Chet, 1968). **In vitro** results in the present work with urea and ammonium nitrate, supports the view of direct inhibitory

Fig. 5.5: Effect of inorganic soil amendments on the yield of soybean under field condition.

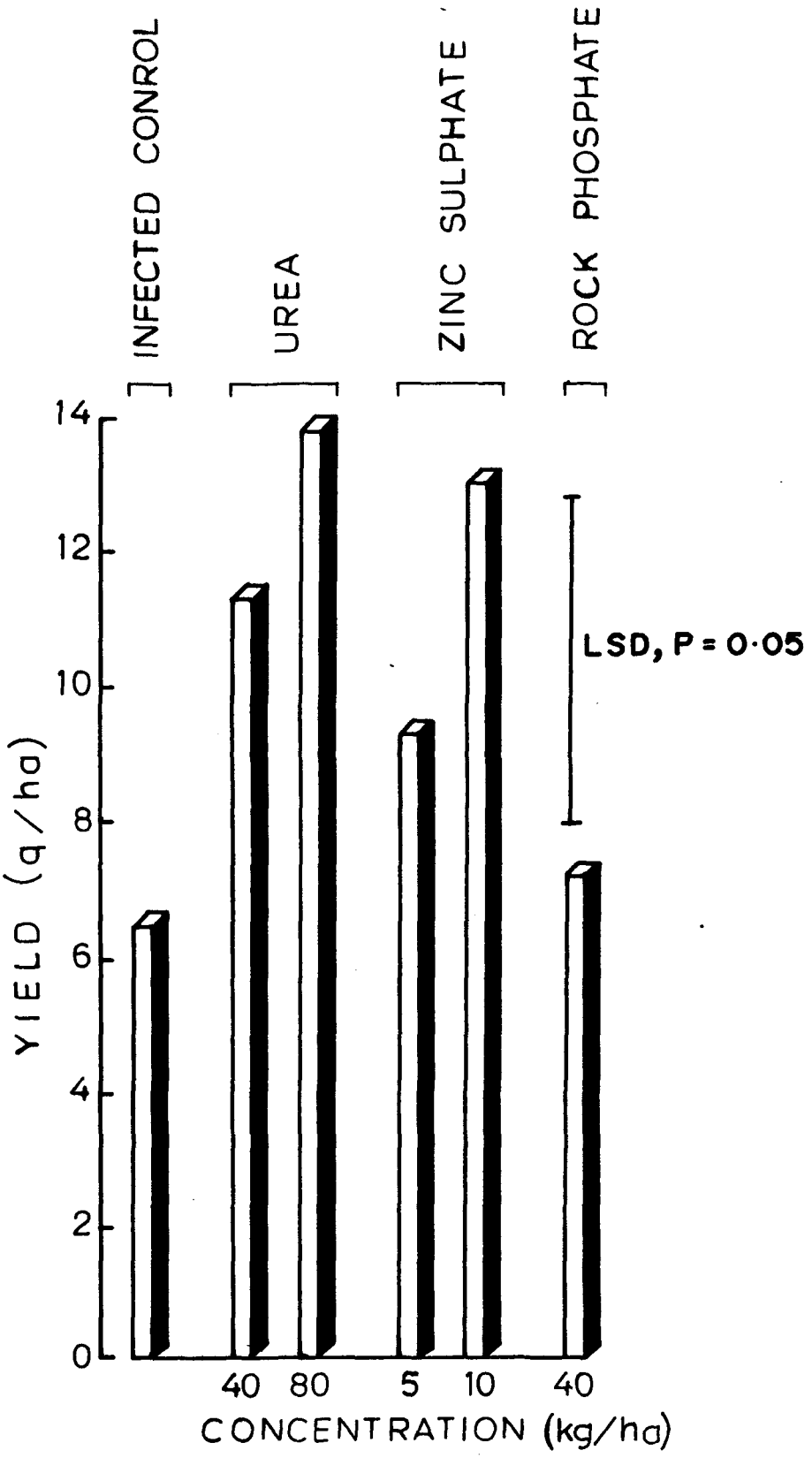

INFECTED CONROL
UREA
ZINC SULPHATE
ROCK PHOSPHATE
14
12
10
8
6
4
2
0
YIELD (q/ha)
LSD, P = 0·05
40
80
5
10
40
CONCENTRATION (kg/ha)

FIG. 5·5

effect of the chemicals on the sclerotial germination and mycelial growth.

Viability of the resting structures in soil, decreased considerably with the increase in concentration of the chemicals used and with the incubation period. Urea amendment stimulated the sclerotial germination resulting in the recovery of empty sclerotial shell from the soil. This suggests that the effect of urea in soil is direct and helps in the eruptive germination of the sclerotium, which is otherwise under an "imposed dormancy" due to soil fungistatic effect. Other amendments also showed reduced sclerotial survibality, possibly due to their toxic effect in the soil as evidenced from **in vitro** results. It is also known that the inorganic soil amendments may influence the disease severity through their effect on the germination of pathogen propagules and mycelial growth in soil or on the host surface. They may also affect the soil microorganisms other than the pathogen. The increased population of the antagonists also might be instrumental in causing a reduction of the **S. rolfsii** population and subsequent reduction in disease severity. Aycock (1966), also reported that the nitrogenous fertilizers reduced the disease severity caused by **S. rolfsii** in various crop plants.

Higher concentrations of urea and ammonium nitrate were found to be toxic to soybean seed germination and radicle

growth, which suggests that the concentration of the chemicals used were not suitable for the plant growth. Experiment **in vivo** also showed poor growth of the seedlings with above mentioned concentrations of the chemicals.

In the present work, an increased population of the antagonistic soil microflora (i.e. actinomycete and **Penicillium** spp.) was observed as a result of calcium nitrate soil amendments. Similar results were also reported by Dutta and Isaac (1979a), where a reduction in disease severity caused by **Verticillium dahliae** to antirrhinum has been correlated to the increase population of actinomycete and **Trichoderma** spp. in the soil following inorganic amendments (i.e. calcium nitrate and combined NPK) to soil. Urea was found to be inhibitory to the fungal population, whereas, ammonium nitrate, rock phosphate and $ZnSO_4$ increased the same considerably. Increase in the fungal population in soil due to inorganic amendments was also reported by Waksman (1922) and Kaufman and Williams (1964). The present findings corroborates the above.

Although, increased actinomycete and fungal population was observed in the amended soil, in rhizosphere a total reduction in microbial population (excepting rock phosphate) was observed, which suggests that the inorganic amendments change the exudation pattern of the host plant causing a change in the rhizosphere population. Increased bacterial population following inorganic amendments have also been

reported by Vraný **et al** (1962). They have observed that the foliar application of urea increased the bacterial population in wheat rhizosphere.

Dominance of Aspergilli, Penicillia, Mucorales in soil and rhizosphere suggests that the disease control achieved in the present work may possibly be due to the microbial activity in the soybean rhizosphere. Reddy (1959) also reported that the foliar spray of urea promoted selectively the growth of a particular species of **Penicillium**, while Agnihotri (1964) found preferential increase of Aspergilli. It was also observed by Kaufman and Williams (1964) that nitrogen fertisation affects the particular genera of fungi (i.e. **Penicillium** spp.). They further observed that the mineral fertilisation significantly affected soil fungus population, but that, as previously stated, nitrogen fertilisation had a greater effect on the individual soil fungi. In the present investigation, dominance of certain genera might have created an antagonistic environment surrounding the pathogen (i.e. **S. rolfsii**), resulting in decreased disease severity in soybean plants.

Nitrogenous fertilizer amendment to soil increased **S. rolfsii** population in the initial stage, thereby increasing pre-emergence of seed rot. Chattopadhyay and Dickson (1960) and Vargas (1972) also have reported that the excess nitrogen increased the brown spot disease of rice caused by **Helminthosporium**

oryzae. The increased disease severity has been reported by them to be due to the increased exudation of nitrogenous compounds i.e. amino acids, from host under excess nitrogen fertilisation which stimulated the pathogen (Chattopadhyay, 1964). A similar conclusion was also drawn by Hobbs and Water (1964) working on the susceptibility of **Chrysanthemum** to **Botrytis cinerea** under high concentration of nitrogen. According to them, an increased concentration of nitrogen in the host tissue served as a better medium for the growth of the pathogen. It was also reported that the nitrogen fertilizer increased the susceptibility of egg plant (**Solanum melongena**) to **Verticillium** wilt disease (Sivaprakasam and Rajagopalan, 1974). Lower concentration of nitrogen in the form of urea decreased susceptibility with delayed symptom expression compared to higher concentration. In the present work, an increased rate of application of urea, calcium nitrate and ammonium nitrate resulted an increase in **S. rolfsii** population, immediately after the soil was amended, resulting in an increased pre-emergence of seed rot. The population subsequently declined which suggests that the nitrogenous fertilizer should be applied much earlier to sowing of the soybean seeds. Late sowing and early urea amendment will possibly reduce the inoculum potential in the process and subsequently a reduction of disease severity may occurs.

Calcium nitrate amendment stimulated actinomycete in soil and bacteria in soybean rhizosphere which might have created an antagonistic environment for the pathogen, resulting a reduction in disease severity. Punja **et al** (1985) also mentioned that increased level of calcium in the carrot tissue following application of calcium nitrate or sulphate, may provide control of disease under low disease pressure. A reduction in disease severity with zinc sulphate amendment conforms with the work of Mehrotra and Claudius (1973), where they have observed an increased disease control of lentil with an increased concentration of $ZnSO_4$ applied as foliar spray.

It is known that the phosphatic rocks can be used for increased crop production in acidic soil. The reserves of insoluble phosphates in soil generally remain unavailable to higher plants unless they were converted into soluble forms by some agents. Soil fungi like **Penicillium pinophilum** and **Aspergillus niger** were found to be more efficient than bacteria (**Bacillus cereus** and **Pseudomonas fluorescens**) in phosphate solubilization (Venkates Worlu **et al**, 1984). Rastogi **et al** (1976) observed maximum availability of phosphorus from Mussoarie rock phosphate. It was also reported earlier that Super phosphate drastically reduced the fungal population in antirrhinum rhizosphere and to some extent reduced the disease severity caused by **Verticillium dahliae** (Dutta and

Isaac, 1979 b). In the present work, rock phosphate increased the microbial population (fungi, actinomycete, bacteria) and decreased actinomycete in the rhizosphere. Emmimoth and Rangaswami (1971) also observed that a significant change in the number and quality of bacteria, fungi and actinomycetes occurs under the influence of heavy doses of fertilizers.

Soybean seed production under field condition is known to be influenced by different factors i.e. different strains of **Rhizobium japonicum**, temperature, rainfall, supply of nitrate nitrogen in soil and also soil fertility (de Mooy **et al**, 1973). Nitrate nitrogen is essential for the vigorous growth of the plant, but excess of it is found to be detrimental to yield, as it directly affects the symbiotic nitrogen fixation capability (Herper, 1974). He further reported that the soybean generally absorbs nitrogen in the form of NH_4^+ during the later stage of seed development but in case where NH^+_4 was the primary source, reduction in yield occurred. In the present work, urea and ammonium nitrate soil amendment reduced the yield possibly because of the fact that these nitrogen forms are available at the initial stage of soybean plant growth only. Calcium nitrate increased the yield partially but higher concentrations (i.e. 0.5% w/w) appeared to be detrimental to soyean seed productions, probably due to inhibited symbiotic nitrogen fixation. Similar results were also obtained by Herper (1974) and Stone **et al** (1985), where a

negative soybean yield response under higher amount of residual nitrate nitrogen was recorded. In the present work, no significant increase in plant growth and yield in general over control was observed possibly due to the limitations of pot culture experiment.

Urea (40, 80 kg/ha) and $ZnSO_4$ (10 kg/ha) gave the best effect on the growth of soybean plant under field condition. Rate of seedling rot in the initial stage, however, was higher with urea (80 kg/ha) which can be correlated with the increase in sclerotial population in soil. Although, $ZnSO_4$ showed an increase in sclerotial population in the soil, but this chemical possibly changed the host metabolism, affecting the susceptibility, resulting in the decrease of disease severity. It was also observed that the higher concentration of $ZnSO_4$ (10 kg/ha) reduced the disease compared to lower concentration (5 kg/ha). Similar results were also obtained by Singh and Aggarwal (1979), where they observed a negative correlation of disease severity with the concentration of zinc applied but a positive relationship has been observed with the levels of phosphorus in soil in case of downy mildew of pearl millet.

Soybean seed yield was however, higher in all the treated plots which is contrary to the results obtained in the pot experiment. However, it is well known that the proper spacing between the seedlings in essential for better growth

and yield. In the pot experiments, lack of space and competition for nutrients might have caused the poor growth and yield. In addition to this, concentration of the fertilizers also played a major role. The amount of fertilizers used in the field were much less compared to pot experiments. An inhibitory effect on the plant growth possibly could be due to the higher amount of fertilizers, which directly reflected on the poor yield produced in such treatments.

CHAPTER IV

EFFECT OF FOLIAR APPLICATION OF NITROFURANS ON THE RHIZOSPHERE MICROFLORA, DISEASE DEVELOPMENT, PLANT VIGOUR AND YIELD IN SOYBEAN PLANTS GROWN IN SCLEROTIUM ROLFSII INFESTED SOIL

INTRODUCTION

In the middle of the nineteenth century it was proved that the plant excretes certain chemical compounds (Sachs, 1860) through their root system into the surrounding soil, and this root excretions make the surroundings a very favourable medium for the activity of soil micro organisms (Hiltner, 1904). Hiltner observed greater microbial accumulation in the soil around the root system than elsewhere in the same soil, which he termed as 'rhizosphere'. It is from the rhizosphere that higher plants obtain necessary nutrients and it is into the rhizosphere that they excrete byproducts of their growth. Due to physiological function of the root, not only the physical and chemical properties of the rhizosphere soil changes, but the rhizosphere microflora population also altered, so that in some cases a beneficial microflora may result, whilst in other cases, a detrimental one could be predominant, affecting the higher plants.

Many antibiotics and broad spectrum antibiotic like chemicals are absorbed by the plant and then translocated systematically within the plants (Crowdy and Pramer, 1955; Goodman, 1962). Nitrofuran derivatives are known to have broad spectrum antibiotic like properties and are widely used as antibacterial substance in the cure of human diseases. Although, antibacterial action of nitrofurans are known, their antifungal action has not been studied so far with reference

to plant disease control. It has been reported by many workers that antibiotics may change the host metabolism, thereby affecting plant disease even if the antibiotic is not fungistatic against the causal organism **in vitro** (Pramer **et al**, 1956). Other factors, which might play an important role in the mode of disease control are, rate of absorption and translocation within the host, which changes the chemical composition of the antibiotics after uptake and stability or persistence of the chemical within the plants (Dekker, 1963).

In the present work, an attempt has been made to explore the possibility of using nitrofurans in the control of foot rot disease of soybean caused by **Sclerotium rolfsii** Sacc. Particular attention was paid to examine the rhizosphere microflora after foliar application in relation to the disease development, plant vigour and yield in the soybean plants, grown in the **S. rolfsii** infested soil.

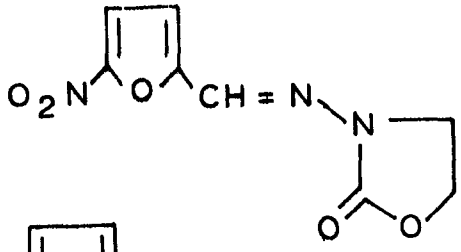
MATERIALS AND METHODS

Nitrofurans used

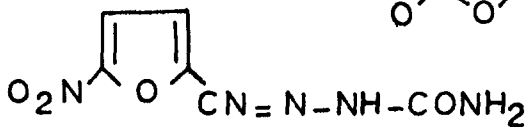
Three nitrofuran derivatives viz. furazolidone, nitrofurazone and nitrofurantoin obtained from commercially available medicine viz. furoxone, furacin and furadantin respectively were used. These were sparingly soluble in water and their solubility increased on slightly heating with acetone.

Chemical structure of nitrofurans

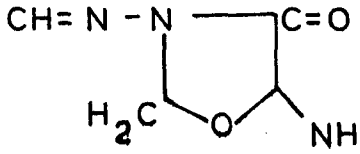
1. Furazolidone:



2. Nitrofurazone:



3. Nitrofurantoin:



Concentrations used

Each nitrofurans under test was calculated to give concentrations of 100, 500 and 1000 µg/L. Since higher concentration is insoluble, 1 ml acetone was added to dissolve the required amount while preparing 1000 µg/L dilution. A control set was also prepared with 1 ml of acetone only (without any nitrofuran).

Fungitoxicity

Subsequently, different concentrations of the chemicals used were made by adding the required amount to the sterilised PDA medium kept at 40±1°c, mixed thoroughly and was poured into petri dishes (9 cm diameter) in triplicate. Inoculum discs (4 mm) of **B. rolfsii**, cut from the periphery of vigorously growing culture were placed in the centre and incubated at

at 25±1°c in dark. Radial expansion was recorded after required period of incubation (i.e. 24, 48, 72 and 96 hours).

Toxicity of nitrofurans on sclerotium was assessed by incubating them in the aqueous solution of the test chemicals, for seven days. The percentage of germination was recorded following the method of Agnihotri **et al** (1975) as described earlier (page 41).

Survibality of Sclerotium

Survival ability of sclerotium was assessed by incubating them in soil drenched with different dilutions of nitrofurans (i.e. 100, 500, 1000 µg/L), separately in replicates. The moisture level of the soil was maintained at 50% by adding aqueous solution of the respective nitrofurans throughout the experiment. Sclerotia were harvested periodically (i.e. after every 15 days) and their germinability was recorded following the method of Agnihotri **et al** (1975) as described in page 41.

Soybean seed germination

Different concentrations of nitrofurans were prepared in distilled water. In case of higher concentration (i.e. 1000 µg/L) of all the chemicals, 1 ml of acetone was added for better solubility. Soybean seeds were placed in the moist chamber containing different concentrations of the nitrofurans

and were incubated. Percentage germination and radicle growth was calculated as described earlier (page 35).

Phytotoxicity

Different dilutions of the nitrofurans described earlier were prepared with Knop's normal solution. Visual observation of phytotoxicity to the one month old soybean seedlings were recorded after 48 hours of root dip treatment.

Pathogenicity test and mode of chemical treatment

Pathogenicity test was carried out by growing the plant from seeds in **S. rolfsii** infested natural soil. Each chemical under test was dissolved in water (as described earlier) to give concentrations of 100, 500, 1000 µg/L and the solution was sprayed separately over one month old soybean seedlings. While spraying, soil was covered with thick Polyethelene sheet, to avoid the contact of the chemicals with soil. Tap water was sprayed on the seedlings which served as control. Fourty seedlings were used in four replicates for each treatment.

Rhizosphere analysis

After fifteen days of foliar spray, ten seedlings from each treatment were uprooted gently without disturbing the roots along with adhering soil. The rhizosphere analysis were made following the method as described earlier (page 39).

Total microbial population and percentage of relative abundance of major groups of rhizosphere fungi was also recorded.

Assessment of disease severity

Number of seedlings infected in each treatment/week was recorded and the infection percentage was calculated.

Effect on plant height

Plant height (cm) was measured weekly till they achieved their maximum height following application of nitrofurans to foliage.

Effect on yield

Yield was determined by taking the mean weight (gm) of seeds from plants of each treatment/control.

RESULTS

Fungitoxicity

Among the nitrofurans, furazolidone at all concentrations used (100, 500, 1000 µg/L) completely inhibited the mycelial growth of **S. rolfsii in vitro.** Nitrofurantoin also completely inhibited the growth at higher concentration only (i.e. 1000 µg/L), while nitrofurazone did not show much

inhibitory effect (Table 4.1) (Plate 12). Germination of sclerotia have also been reduced with the increase in concentration of all the nitrofurans tested. The germination was less in nitrofurantoin treatment compared to furazolidone (Table 4.1). Survibality of the sclerotium in soil decreased with the increase in concentration and incubation period. Furazolidone (1000 µg/L) completely eliminated the viable sclerotia after 30 days, while others showed comparatively poor germination (Table 4.1).

Seed germination and phytotoxicity

The effect of nitrofurans on soybean seed/seedlings have shown in Table 4.2. Although, seed germination was not inhibited (100% germination) by the nitrofurans tested, a gradual reduction in radicle growth with the increase in the concentration of the chemicals were observed. None of the nitrofuran derivatives tested showed any phytotoxic effect, excepting higher concentration of nitrofurazone (500 and 1000 µg/L), where mild chlorosis in the leaf margin was observed after 24 hours of exposure.

Effect on the rhizosphere

A drastic reduction in fungal and bacterial population was observed in the rhizosphere of the foliar sprayed soybean plants, whereas actinomycete population was found to be increased significantly (Table 4.2). Maximum reduction in fungal population

TABLE 4.1 : Effect of nitrofurans on the mycelial growth and sclerotial germination.

Nitrofurans	Concentration (µg/L)	Radial expansion(mm)[1] (Period of Incubation(Days))		Sclerotial germination(%)				
				In aqueous solution(after 7 days)[2]	In soil[3] (Period of Incubation(Days))			
		3	5		15	30	45	60
Furazolidone	100	-	-	60.00	57.14	42.80	38.80	29.40
	500	-	-	46.70	30.00	16.70	10.00	-
	1000	-	-	36.70	20.00	20.00	-	-
Nitrofurazone	100	52±3.22	89±0.07	100.00	68.40	41.60	35.70	25.00
	500	49±2.65	88±0.08	90.00	46.70	31.25	15.30	10.00
	1000	50±2.65	86±0.06	86.70	33.30	12.50	10.00	7.69
Nitrofurantoin	100	46±1.16	85±0.08	53.30	58.30	38.90	25.00	20.00
	500	33±2.38	55±0.06	33.30	44.40	28.50	27.70	15.00
	1000	-	-	33.30	46.70	31.50	15.00	8.33
Control (without treatment)	-	45±2.89	90±0.00	100.00	100.00	100.00	100.00	100.00

1) Mean of three replicates with ±S.E.; (-) Nil (no growth/germination).
2) Observation based on 30 sclerotia in each case.
3) Calculation based on recovered sclerotia in each case.

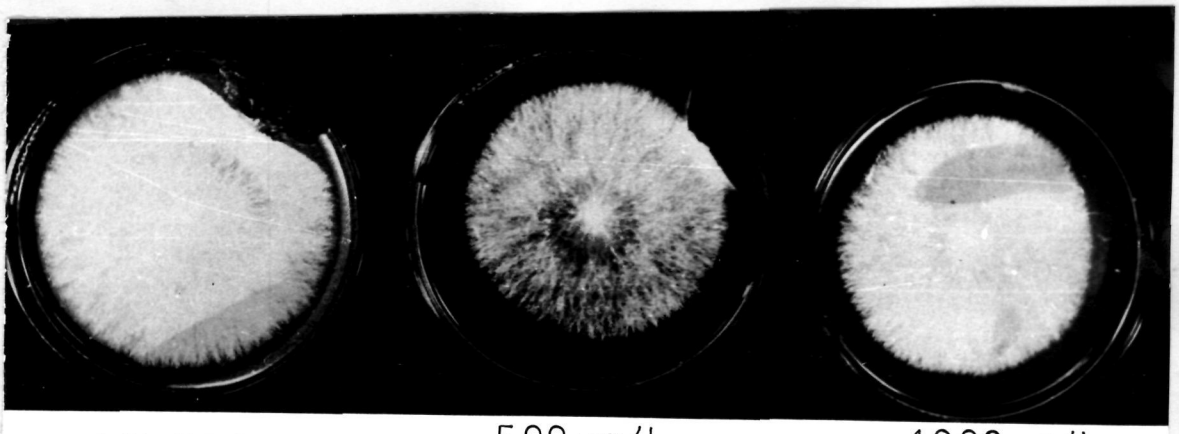
TABLE 4.2 : Effect of nitrofurans on soybean seed germination, seedling growth and on the rhizosphere microflora in Pathogen infested soil.

Nitrofurans	Concentration (µg/L)	In vitro test			In vivo test					
		Seed germination (%)	Mean radicle length ±S.E. (mm)[1]	Phytotoxicity[2]	Rhizosphere microorganisms in thousands g^{-1} dry soil[3]			Rhizosphere effect (RS/CS)[4]		
					Fungi (10^4)	Actinomycete ($2x10^4$)	Bacteria ($3x10^4$)	Fungi	Actinomycete	Bacteria
Furazolidone	100	100	43±0.54	-	26	671.00	253.00	1.36	5.59	0.63
	500	100	41±0.96	-	14	538.00	206.00	0.74	4.48	0.51
	1000	100	39±0.87	-	12	406.00	200.00	0.63	3.38	0.50
Nitrofurazone	100	100	35±0.15	-	60	419.00	269.00	3.16	3.49	0.67
	500	100	30±0.40	+	23	565.00	193.00	1.20	4.71	0.48
	1000	100	23±0.04	+	12	400.00	165.00	0.63	3.33	0.41
Nitrofurantoin	100	100	39±0.87	-	45	453.00	265.00	2.36	3.78	0.66
	500	100	35±0.15	-	36	451.00	266.00	1.89	3.76	0.66
	1000	100	30±0.41	-	34	505.00	241.00	1.79	4.21	0.60
Control rhizosphere soil	-	100	82±0.05	-	37	270.00	400.00	1.94	2.25	1.00
Control soil	-	-	-	-	19	120.00	400.00	-	-	-
Significance by F test (1%)	-	-	-	-	Yes	Yes	Yes			

1) Calculation based on 25 seeds in each case.
2) (-) Non toxic; (+) Slightly toxic.
3) Mean of three replicates in each case.
4) Rhizosphere soil/control soil.

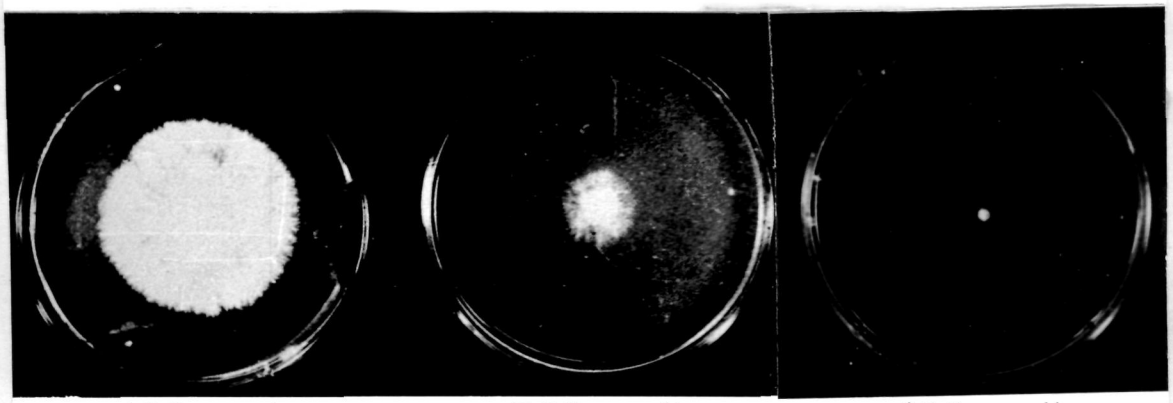
Plate 12: Showing the effect of nitrofurans on the growth of **Sclerotium rolfsii in vitro.**

PLATE-12



CONTROL 500 µg/l 1000 µg/l

NITROFURAZONE



100 µg/l 500 µg/l

NITROFURANTOIN

100 µg/l

FURAZOLIDONE

was recorded with furazolidone followed by nitrofurazone treatment, while higher concentration of nitrofurazone (1000µg/L) decreased the bacterial population considerably.

Increased population of Aspergilli and mucorales due to nitrofuran spray was observed in the soybean rhizosphere, whereas penicillia was found to be decreased drastically. Among the four **Aspergillus** spp., **A. candidus** and **A. niger** were found to be dominant in the rhizosphere. **Rhizopus stolonifer** and **Humicola** sp. were also found to be present in almost all the chemically treated soybean rhizosphere. An increased percentage of relative abundance of these fungi was observed in the treated plants, compared to non sprayed control (Table 4.3). Occurrence of **Fusarium** spp. and **Trichoderma** spp. in the rhizosphere was also observed after the foliar spray of nitrofurans. Other species included **Cladosporium** sp., **Cephalosporium** sp., **Gliocladium** sp. **Geotrichum** sp., **Verticillium** sp. and red yeast, whose percentage of relative abundance was also found to have slightly increased compared to non treated infected control. A total of twentyseven rhizosphere fungi belonging to fourteen genera and an unidentified white mycelium have been isolated (Table 4.4).

Assessment on disease severity

It was observed that in chemically treated plants the disease symptom appeared at a much later stage i.e. 15 days

TABLE 4.3 : Percentage of relative abundance of the predominant genera of fungi in the rhizosphere of soybean in Pathogen infested soil.

Nitrofurans	Concentration (µg/L)	Aspergilli	*Fusarium* spp.	Mucorales	Penicillia	*Trichoderma* spp.	Other spp.
Furazolidone	100	8.50	7.00	16.90	23.90	-	43.70
	500	10.00	4.30	18.60	17.20	-	49.90
	1000	19.30	-	8.10	19.30	4.80	48.20
Nitrofurazone	100	3.80	-	46.70	6.00	3.00	41.10
	500	8.50	6.40	25.60	17.10	-	42.40
	1000	27.20	-	13.60	-	4.50	54.50
Nitrofurantoin	100	21.10	-	10.50	-	5.30	63.50
	500	6.30	12.50	62.40	-	-	18.80
	1000	14.30	25.00	35.80	-	3.60	21.40
Control rhizosphere soil	-	6.30	-	3.12	50.00	-	40.77
Control soil	-	15.15	-	30.35	33.33	3.03	18.23

TABLE 4.4 : Effect of Nitrofuran foliar spray on the percentage of relative abundance of rhizosphere fungi under infested condition.

Soil & rhizosphere fungi	Control (without treatment)	Control rhizos-phere (without treatment)	Furazolidone			Nitrofurazone			Nitrofurantoin		
			100	500	1000	100	500	1000	100	500	1000
Absidia repens van. Tieghem.	6.06	-	-	-	-	-	-	-	-	-	-
Absidia sp.	-	-	-	-	-	16.70	8.52	-	-	6.30	-
Aspergillus candidus Link ex. Fries.	6.06	3.12	8.40	5.60	9.60	3.30	-	13.62	-	-	10.80
A. flavipes Thom & Church	-	-	-	-	-	-	-	-	15.90	-	-
A. niger van. Tieghem.	-	-	-	4.20	4.80	-	8.52	13.62	5.30	6.30	-
A. versicolor (Vuillemin) Tiraboschi.	9.09	3.12	-	-	4.80	-	-	-	-	-	3.60
Cephalosporium acremonium Corda.	-	6.24	-	14.00	9.60	10.00	10.65	22.70	10.60	6.30	10.80
Cladosporium herbarum Link ex. Fries.	-	-	-	7.00	8.00	-	6.30	-	5.30	-	-
Fusarium oxysporum	-	-	8.40	4.20	-	-	6.39	-	-	12.60	10.80
F. solani	-	-	-	-	-	-	-	-	-	-	14.40
Geotrichum sp.	-	6.24	28.20	15.40	14.40	26.70	21.30	31.78	21.20	-	10.80
Gliocladium catenulatum Gilman & Abbott.	-	-	-	-	-	-	-	-	-	12.60	-
Humicola sp.	-	-	11.20	12.60	1.60	20.00	12.78	-	-	18.90	10.80
Mucor hiemalis Wehmer.	-	3.12	-	-	-	-	-	-	-	25.20	14.40
Mucor sp.	9.09	-	-	-	-	-	-	-	-	-	-
Penicillium brefeldianum Dodge.	-	3.12	5.60	11.20	6.40	3.30	12.78	-	-	-	-
P. chrysogenum Thom.	12.12	-	-	5.60	-	3.30	-	-	-	-	-
P. granulatum Bainier.	-	3.12	-	-	4.80	-	-	-	-	-	-
P. nigricans Bainier.	-	-	5.60	-	-	-	-	-	-	-	-
P. oxalicum Thom.	9.09	-	-	-	-	-	-	-	-	-	-
P. rubrum Stoll.	9.09	24.96	11.20	-	8.00	-	-	-	-	-	-
P. vermiculatum Dangeard.	3.03	18.72	-	-	-	-	4.26	-	-	-	-
Rhizopus stolonifer (Ehrenb ex. Link) Lind 1913.	15.20	-	5.60	5.60	6.40	10.00	4.26	13.62	10.60	12.60	10.80
Trichoderma koningii oudemans.	-	-	-	-	-	3.30	-	4.54	5.30	-	-
T. viride pers Ex. Fries.	3.03	-	-	-	4.80	-	-	-	-	-	3.60
Verticillium sp.	3.03	-	-	1.40	-	-	-	-	-	-	-
Yeast (red)	15.20	18.72	16.80	11.20	16.00	3.30	-	-	26.50	-	-
Unidentified	-	9.36	-	1.40	-	1.67	4.26	-	-	-	-

Nitrofurans applied to foliage at a rate of 100, 500, 1000 ug/L to soybean plant.

later than that of nontreated infected control.

Of the three nitrofurans tested, nitrofurantoin was the most effective chemical in controlling the disease as it caused delay in symptom expression and reduced the severity, considerably. Furazolidone and nitrofurazone also reduced the disease by more than 50% over nontreated infected control (Fig. 6.1).

Effect on plant height

Increased plant height was observed in case of nitrofurazone (100, 500 µg/L) and furazolidone treatment, at all the concentrations used (Fig. 6.1). Higher concentration of nitrofurazone and nitrofurantoin (1000 µg/L) was however found to be toxic to the plants, as they adversely affected the growth.

Effect on yield

Yield was always high in treated plants (Fig. 6.2) excepting nitrofurantoin (1000 µg/L) compared to nontreated infected control. Furazolidone gave comparatively better yield at all the concentrations used (Plate 13). Higher concentration of nitrofurantoin showed poor yield, although it delayed the expression of disease symptom and reduced the severity.

Fig. 6.1: Effect of foliar application of nitrofurans (A. Furazolidone, B. Nitrofurazone, C. Nitrofurantoin) on the growth/disease development in soybean caused by **Sclerotium rolfsii.**

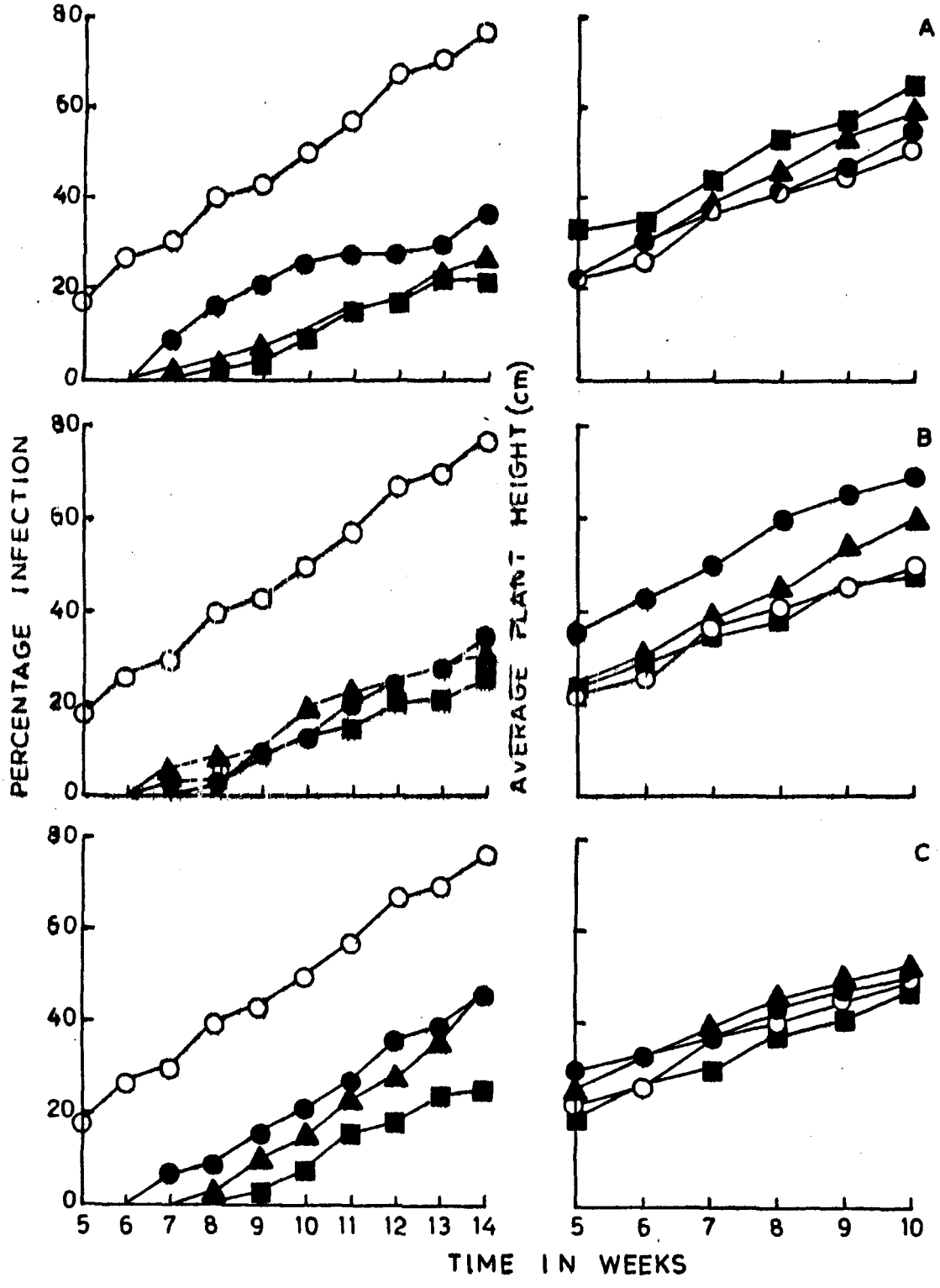

PERCENTAGE INFECTION
AVERAGE PLANT HEIGHT (cm)
TIME IN WEEKS
A
B
C
0
20
40
60
80
5
6
7
8
9
10
11
12
13
14

FIG. 6·1

Fig. 6.2: Effect of nitrofuran foliar sprays on the soybean yield.

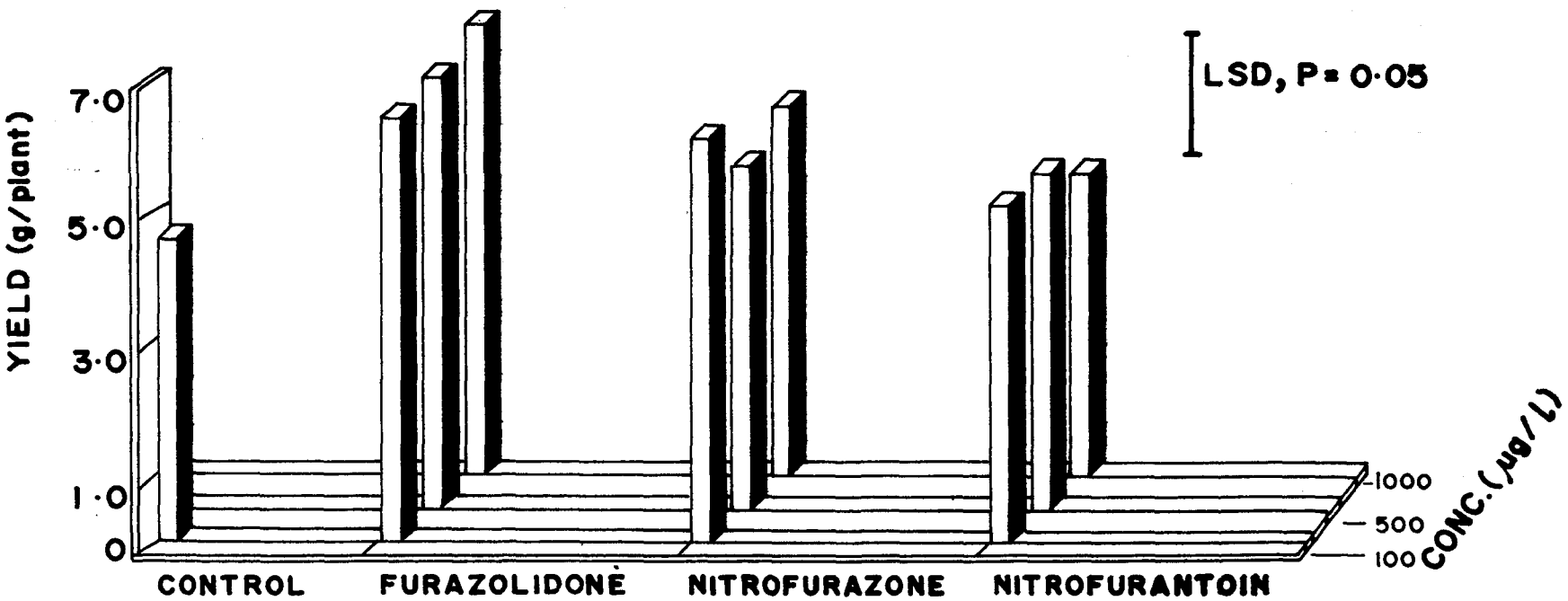

YIELD (g/plant)
7·0
5·0
3·0
1·0
0
LSD, P = 0·05
1000
500
100
CONC. (µg/L)
CONTROL
FURAZOLIDONE
NITROFURAZONE
NITROFURANTOIN

Fig. 6·2

Plate 13: Foliar spray of furazolidone induced better disease control and growth of soybean plants compared to infected control.

PLATE-13

DISCUSSION

Treatment of plants with antibiotic/antibiotic like substances are commonly employed to control plant diseases and to have a better crop yield. The mode of action seems to be due to the fact that the antibiotic/antibiotic like substances treatment affects the physiology of the treated plants, which consequently affect the rhizosphere microflora with a reduction in disease severity and increased yield.

All the nitrofurans tested showed inhibitory effect against the growth of **S. rolfsii in vitro.** Furazolidone and higher concentration of nitrofurantoin completely inhibited the growth of the test pathogen, while nitrofurazone did not have any affect. 50% reduction in sclerotial viability after 15 days of treatment and complete elimination after 30 days in case of furazolidone (1000 µg/L) suggests their fungitoxic nature.

In the present study, nitrofuran treated plant showed reduction in disease severity. It was reported earlier that the antibiotics are taken up and systematically translocated within the plant (Crowdy and Pramer, 1955). It has been established that antibiotics may change the host metabolism and affect plant diseases even if the chemical is not fungistatic against the causal organisms **in vitro** (Pramer **et al**, 1956). Slakins **et al** (1964) reported that the application of $C^{14}O_2$

on the leaves of white pine, released more than 35 radioactive compounds in the root excretion. It is understood, therefore, that the chemicals applied on the leaves, enter into various metabolic pathways of plants and are excreted through the roots along with other compounds. In the present work, although nitrofurazone and nitrofurantoin were less effective against **S. rolfsii in vitro**, they were found to reduce disease severity, considerably. These chemicals may have changed the host metabolism resulting the disease control, but had an adverse affect on the plant growth and yield. It has also been reported earlier that the soil-borne plant diseases could be controlled by fungistatic and non fungistatic chemicals, which induce disease resistance and improve yield potential in the susceptible host plants (Dutta, 1980; Dutta and Isaac, 1981). Similar results were also observed with furazolidone (fungitoxic) and nitrofurazone (non fungitoxic), both of which increased disease resistance to a great extent with an increase in yield.

It was further observed that the fungal and bacterial population in the rhizosphere decreased drastically in the nitrofuran treated soybean plants, whereas actinomycete population was stimulated. Decrease in bacterial population in the bean rhizosphere, after foliar sprays of some fungicides has also been reported by Halleck and Cochrane (1950). They had observed qualitative and quantitative changes after

the foliar spray of bordeaux mixture, malachite green and dithane Z-78 in host rhizosphere. It has also been reported earlier that wilt of tomato caused by **Verticillium albo-atrum** was reduced by the foliar spray of organic and inorganic chemicals (Baruah and Dutta, 1978). Reduction of fungi and increase of antagonistic actinomycete population in the rhizosphere due to foliar spray in the present work may be correlated with the decrease in disease severity. It was reported earlier that a reduction of disease severity of antirrhinum plants infected with **Verticillium dahliae** could be correlated with the increase in the population of actinomycete in the rhizosphere (Dutta and Isaac, 1979a).

Most of the fungal species isolated, were common in foliar sprayed and in the control rhizosphere. Total population of fungi together with the total number of species decreased in the foliar treated plant's rhizosphere. Foliar spray increased Aspergilli in particular, in the rhizosphere of the treated plants. Antagonistic **Trichoderma** spp. was found to be present in the rhizosphere of most of the nitrofuran treated plants, while **Penicillium** spp. decreased significantly. These changes in host rhizosphere after the foliar spray seems to be due to the altered host metabolism and exudation pattern through the root system.

Higher concentration of nitrofurazone and nitrofurantoin applied to the soybean plants were found to be toxic but

delayed/suppressed the disease symptom expression. This indicates that the concentration of the nitrofurans used should be considered as an important factor for its possible application as chemotherapeutant. Nitrofurans do not have any toxicity to seed germination, which suggests that the seed dressing with the nitrofurans possibly be effective in controlling the foot rot diseases of soybean caused by **S. rolfsii.**

Reduction in yield in case of nitrofurantoin is possibly due to its toxic effect to soybean seedlings causing defoliation and decreased the size of newly formed leaves after spraying. Ivins and Breminer (1964) suggested that the yield was proportional to leaf area and duration. Harrison (1968) also showed a close correlation between percentage reduction in leaf area and percentage reduction in tuber yield of potato, indicating that the pathogen mainly reduce yield by reducing leaf area and duration of leaves. But in the present work, a delayed/reduced infection rate together with poor yield suggests that, nitrofurantoin might have changed the host metabolism which out-weights the benefit obtained by this chemical. Furazolidone and nitrofurazone reduced disease severity with an increase in the yield, which support their chemotherapeutic activity for the control of foot rot of soybean caused by **S. rolfsii.**

CHAPTER V

EFFECT OF FOLIAR APPLICATION OF ANTIBIOTICS ON THE RHIZOSPHERE MICROFLORA, DISEASE DEVELOPMENT, PLANT VIGOUR AND YIELD IN SOYBEAN PLANTS GROWN IN SCLEROTIUM ROLFSII INFESTED SOIL

INTRODUCTION

Importance of antibiotics in plant disease control has been well recognised for a long time and have played a dominant role in the development of plant chemotherapy. Several attempts have been made to apply antibiotics to control the pathogenic fungi that cause plant diseases and are resistant to or slow to respond to other methods of chemotherapy. Attention was given to understand the chemical structure of the antifungal compounds which might lead to the development of valuable synthetic fungicides.

Antagonism among the microorganisms, and their influence on plant disease control have been well known. Several antifungal antibiotics i.e. endomycin and thiolutin, have been isolated from the strains of **Streptomyces albus** (Taner **et al**, 1950; Gottlieb **et al**, 1951). Gliotoxin was the first antibiotic to be used in plant disease control. Isolation and purification of this antibiotic was done before the discovery of penicillin.

Many antibiotics are reported to be translocated systematically and therefore play important role in the control of vascular and soil-borne plant diseases, causing a change in the rhizosphere environment.

In the present work an attempt was made to study the effect of antibiotic foliar spray on the rhizosphere

microflora in relation to disease severity, plant vigour and yield in soybean plants grown in **S. rolfsii** infested natural soil.

MATERIALS AND METHODS

Five different antibiotics viz. (1) Streptomycin (2) Thiolutin (3) Penicillin (4) Actidione (5) Chloramphenicol were used.

1. **Streptomycin** is a systemic antibiotic, known to be active against a large number of bacteria. However, it has relatively little effect on the anaerobic bacteria and fungi. The use of streptomycin as a seed disinfectant has been extensively explored with considerable success.

2. **Thiolutin** is readily extracted from several strains of **Streptomyces albus**. It inhibits both gram negative and gram positive bacteria and many fungi. The brilliant yellow needle shaped crystals, sparingly soluble in water, are more soluble in organic solvements. Ultra violet absorption maxima are at 314 and 364 μ. The compound contains carbon, hydrogen, nitrogen and sulphur. Thiolutin was kindly supplied by Charles Pfizer and Co., New York.

3. **Penicillin:** This broad spectrum antibiotic isolated from **Penicillium notatum**, widely used as antibacterial medication.

It is also obtained from **P. chrysogenum**. Penicillin is active against gram positive bacteria and is of low toxicity to man. It is synthetically prepared and obtained commercially.

4. **Actidione** (Cyclohexamide) is available commercially. It is produced simultaneously with streptomycin from **Streptomyces griseus**, and has marked antifungal properties **in vitro**, although it does not have antibacterial activity. Chemically it is β-(2-(3,5 dimethyl-2-Oxycyclohexyl)-2-hydroxyethyl) gluterimide.

5. **Chloramphenicol** has broad spectrum antibacterial activity and has been widely tested in plant disease control. It is synthetically prepared and can be obtained commercially.

It is difficult to dissolve thiolutin in water at higher concentration, hence the antibiotic was first dissolved in a small quantity of acetone (1% only) and water was added to give the required dilution. The same quantity of acetone was also added to the control solution. The concentration of antibiotics used in the present study were 20, 40, 80, 100 µg/L.

Effect of antibiotics on the growth, sclerotial germination of **S. rolfsii** and on soybean seed germination **in vitro** were determined following the method as described earlier (page 34-35).

Soybean seedlings were raised in the **S. rolfsii** infested natural soil (at ICAR farm, Barapani). To one month

old seedlings, different concentration (20, 40, 80, 100 µg/L) of antibiotics were sprayed following the procedures as described before (page 128).

Rhizosphere analysis, assessment of disease severity, plant vigour (i.e. height) and yield were estimated following the methods as described earlier (page 39-42).

RESULTS

Fungitoxicity

Among the antibiotics used, actidione and thiolutin (Plate 14) completely inhibited the growth of **S. rolfsii** even at lower concentration **in vitro** (i.e. 20 µg/L). Others also showed inhibitory effect but only with higher concentrations (80 and 100 µg/L) (Table 5.1). A considerable decrease in germination of sclerotia in aqueous solution of antibiotic was observed, of which thiolutin and actidione produced highest inhibition (Table 5.2).

In soil, almost all the concentrationsof the antibiotics used showed fungitoxic effect and approximately 50% of the sclerotia found to remain viable upto 15 days of incubation in soil. The viability of the sclerotia in soil decreased with the increase in the concentration of the chemicals and with the incubation period. Total loss of viable sclerotia after 60 days of incubation was recorded with the higher

Plate 14: Showing the effect of thiolutin (20 µg/L) on the growth of **Sclerotium rolfsii in vitro.**

PLATE-14

CONTROL

THIOLUTIN

TABLE 5.1 : Effect of Antibiotics on the growth[1] (Radial Expansion (mm)) of S. rolfsii in vitro.

Antibiotics	Concentration (µg/L)	Period of Incubation (Hours)			
		24	48	72	96
Actidione	20	-	-	-	-
Chloramphenicol	20	19.50±0.90	45.00±1.77	61.25±3.92	78.25±3.68
	40	20.25±1.47	47.50±1.25	53.50±2.38	75.75±1.88
	80	16.50±1.15	37.50±3.75	54.30±2.20	68.30±3.81
	100	18.50±1.02	37.50±2.80	54.75±4.68	63.75±5.40
Penicillin	20	18.70±1.53	36.70±2.57	64.30±4.02	87.30±2.59
	40	17.30±1.89	36.00±2.50	43.30±5.00	65.70±5.72
	80	20.70±1.78	35.70±2.70	45.60±2.57	65.30±2.87
	100	16.70±0.47	35.30±2.75	43.70±3.30	53.30±3.77
Streptomycin	20	15.50±1.82	30.00±1.77	61.25±5.54	90.00±0.00
	40	13.75±1.29	36.70±1.01	62.50±2.79	81.70±4.70
	80	15.00±1.27	30.00±2.36	58.00±2.94	81.70±3.34
	100	16.75±1.35	35.00±3.06	60.25±1.85	75.00±2.36
Thiolutin	20	-	-	-	-
Control (without treatment)	-	26.00±1.46	45.00±2.06	70.25±1.85	90.00±0.00

1) Mean of three replicates ±S.E.; (-) Nil.

TABLE 5.2 : Effect of antibiotics on the survibality of Sclerotium.

Antibiotics	Concentration (µg/L)	Sclerotium Germination(%)				
		In aqueous solution after 7 days[1]	In soil[2] (Period of Incubation (Days))			
			15	30	45	60
Actidione	20	73.30	50.00	41.60	27.30	25.00
	40	53.30	46.70	33.30	26.70	10.50
	80	46.70	46.70	38.50	15.40	6.70
	100	40.00	30.00	10.50	8.33	-
Streptomycin	20	80.00	41.70	38.50	25.00	10.00
	40	73.30	36.25	33.30	20.00	11.10
	80	66.70	33.30	28.60	22.80	8.33
	100	60.00	30.00	26.70	16.70	-
Chloramphenicol	20	66.70	53.30	46.70	33.30	25.00
	40	60.00	33.30	20.00	12.50	6.70
	80	53.30	33.30	12.50	8.33	-
	100	46.70	26.70	20.00	6.70	-
Penicillin	20	73.30	70.00	41.70	30.00	20.00
	40	60.00	50.00	33.30	25.00	15.40
	80	53.30	40.00	26.70	20.00	13.30
	100	53.30	20.00	13.30	6.70	-
Thiolutin	20	46.70	46.70	30.80	20.00	-
	40	33.30	37.50	27.80	15.40	-
	80	26.70	33.30	26.70	13.30	-
	100	26.70	30.00	20.00	6.70	-
Control (without treatment)	-	100.00	100.00	100.00	100.00	100.00

1) Calculation based on 60 sclerotia in each case.
2) Calculation based on recovered sclerotia in each case.

(-) No germination.

concentration of all the antibiotics tested (Table 5.2).

Seed germination and phytotoxicity

Seed germination was found to be inhibited by almost all the antibiotics tested. Comparatively higher inhibition was recorded with actidione and chloramphenicol. Radicle growth was also reduced by all the antibiotics, irrespective of the concentrations used, excepting streptomycin, where it showed stimulatory affect. Actidione, chloramphenicol and thiolutin, at higher concentration (i.e. 80 and 100 µg/L) showed phytotoxic effect. Mild chlorosis in the margin of leaves in the treated plants was also observed (Table 5.3).

Effect on the rhizosphere

Fungal population decreased significantly in the rhizosphere of all the antibiotics treated plants. The inhibition increased with the increase in concentration of the chemicals used. Higher reduction of fungi in the rhizosphere was observed with chloramphenicol spray, considerable reduction in bacterial population was also observed. Whereas actinomycete population showed an increase in all the treatments excepting chloramphenicol (Table 5.3). In general actinomycete population increased with an increase in the concentration of the antibiotics used.

Aspergillus spp., **Fusarium** spp., **Penicillium** spp., Mucorales and **Trichoderma** spp. are the dominant genera of

TABLE 5.3 Effect of antibiotics on the soybean seed, seedlings and rhizosphere grown in **S. rolfsii** infested soil.

Antibiotics	Concentration (µg/L)	In vitro test			In vivo test					
		Seed germination (%)	Radicle length ±S.E.(cm)[1]	Phytotoxicity[2]	Rhizosphere microorganisms[1] in thousands[3] g^{-1} dry soil			Rhizosphere effect (RS/CS)[4]		
					Fungi (10^4)	Actinomycete ($2x10^4$)	Bacteria ($3x10^4$)	Fungi	Actinomycete	Bacteria
Actidione	20	75.00	9.65±2.25	-	12.00	230.00	286.60	0.63	1.92	0.72
	40	60.00	8.78±1.34	-	12.08	285.80	285.80	0.63	2.38	0.71
	80	60.00	5.87±1.54	+	7.00	291.60	252.50	0.37	2.43	0.63
	100	46.70	3.00±0.45	++	6.60	290.00	237.00	0.35	2.42	0.59
Streptomycin	20	100.00	12.38±1.45	-	15.40	294.00	245.80	0.81	2.45	0.62
	40	100.00	12.75±1.13	-	8.75	288.40	210.80	0.46	2.40	0.53
	80	86.60	11.00±0.82	-	5.00	305.00	156.70	0.26	2.54	0.39
	100	80.00	12.80±0.88	-	4.16	323.20	136.70	0.22	2.69	0.34
Chloramphenicol	20	86.60	9.00±1.00	-	11.25	240.00	250.80	0.59	2.00	0.63
	40	66.70	9.86±1.17	-	7.90	229.15	248.30	0.42	1.91	0.62
	80	60.00	8.40±1.30	-	4.20	245.00	255.80	0.22	2.04	0.64
	100	60.00	7.00±1.50	+	3.75	270.80	190.00	0.20	2.26	0.48
Penicillin	20	100.00	13.00±0.88	-	10.00	237.50	290.80	0.53	1.98	0.73
	40	93.30	11.65±1.28	-	8.33	323.40	257.50	0.44	2.70	0.64
	80	93.30	8.45±1.20	-	8.33	387.50	271.70	0.44	3.23	0.70
	100	86.70	8.10±0.05	-	5.80	404.40	243.30	0.31	3.37	0.61
Thiolutin	20	100.00	5.21±0.46	-	18.10	302.50	301.20	0.95	2.52	0.75
	40	100.00	4.90±0.85	-	13.75	353.20	245.00	0.72	2.94	0.61
	80	100.00	4.70±0.49	-	12.50	325.80	239.16	0.66	2.72	0.61
	100	80.00	4.20±0.09	+	10.00	403.20	198.30	0.53	3.36	0.50
Control rhizosphere soil	-	100.00	8.20±0.05	-	37.00	270.00	400.00	1.95	2.25	1.00
Control soil	-	-	-	-	19.00	120.00	400.00	-	-	-
Significance by F test (1%)	-	-	-	-	Yes	Yes	Yes	-	-	-

1) Calculation based on 25 seeds in each case. (2) (-) Non toxic; (+) slightly toxic; (++) toxic. (3) Mean of three replicates in each case. (4) Rhizosphere soil/control soil.

fungi isolated from rhizosphere of almost all the treatments. Chloramphenicol, penicillin, thiolutin and actidione treatment showed an increase in **Aspergillus** spp. population, Mucorales in the rhizosphere increased tremendously while **Penicillium** spp. found to be reduced following antibiotic foliar spray (Table 5.4).

A total of twentynine species belonging to thirteen genera have been isolated from the soybean rhizosphere, of which **Absidia repens, Aspergillus niger, Geotrichum** sp., **Penicillium chrysogenum, Rhizopus stolonifer** and yeast were the most frequently occuring organisms in the rhizosphere (Table 5.5).

Effect on the disease severity

The infection rate was observed to have reduced in all the treatments (Table 5.6). Actidione, chloramphenicol and thiolutin (all the concentrations) delayed the on set of disease, by one week or more. In all the cases, higher concentration of the antibiotics (80 and 100 µg/L) reduced the infection rate and delayed the symptom expression. Compared to other treatments, highest disease control was observed with actidione (20% infection), followed by penicillin and thiolutin (23.3 and 26.7% infection respectively) at 100 µg/L.

TABLE 5.4 : Effect of antibiotic foliar spray on the percentage of relative abundance of major groups of fungi in soybean rhizosphere grown in **S. rolfsii** infested soil.

Antibiotics	Concentration (µg/L)	Aspergilli	Cephalosporium spp.	Cladosporium spp.	Fusarium spp.	Mucorales	Penicillia	Trichoderma spp.	Yeast	Other genera of fungi imperfecti
Actidione	20	24.18	3.45	3.45	10.34	13.79	34.48	3.45	-	6.89
	40	17.24	6.89	-	-	24.12	21.02	3.45	6.89	10.34
	80	17.66	11.76	-	11.76	11.76	17.66	17.66	11.76	-
	100	6.25	-	12.50	12.50	31.25	25.05	12.50	-	-
Streptomycin	20	4.76	-	-	-	33.32	9.52	9.52	23.80	19.04
	40	2.70	-	-	-	21.60	48.60	8.10	5.40	13.50
	80	-	-	-	-	25.00	41.70	16.70	8.30	8.30
	100	-	-	-	37.50	37.50	12.50	12.50	-	-
Chloramphenicol	20	27.23	-	-	-	22.70	27.23	9.09	13.63	-
	40	15.38	-	15.38	-	38.45	30.69	-	-	-
	80	16.64	33.33	-	-	16.64	16.64	16.64	-	-
	100	16.64	-	16.64	-	33.33	16.64	-	-	16.64
Penicillin	20	8.32	-	4.16	16.64	41.64	4.16	8.33	8.33	8.33
	40	6.25	-	-	6.25	56.25	6.25	-	18.75	6.25
	80	10.00	5.00	10.00	25.00	35.00	-	-	10.00	5.00
	100	7.69	15.38	-	7.69	30.76	-	-	23.07	15.38
Thiclutin	20	16.13	-	6.45	9.68	29.03	25.80	-	6.45	6.45
	40	20.67	-	3.44	-	27.56	6.89	-	31.63	10.34
	80	20.00	6.70	10.00	6.70	13.30	20.10	-	13.63	10.00
	100	17.64	-	11.76	11.76	23.52	17.64	5.88	11.76	-
Control rhizosphere soil	-	6.30	6.30	-	-	3.10	50.00	-	18.80	15.67
Control soil	-	15.15	-	-	-	30.35	33.33	3.03	15.20	3.03

TABLE 5.5 : Effect of antibiotic foliar spray on the percentage of relative abundance of rhizosphere fungi under infested condition.

Soil and rhizosphere fungi	Control (without treatment)	Control rhizosphere (without treatment)	Actidione				Streptomycin				Chloramphenicol				Penicillin				Thiolutin			
			20	40	80	100	20	40	80	100	20	40	80	100	20	40	80	100	20	40	80	100
Absidia repens van Tieghem.	6.06	-	-	6.89	-	-	19.04	-	25.00	12.50	-	-	-	-	4.16	18.75	10.00	7.69	3.22	6.89	-	-
Aspergillus candidus Link. ex. Fries.	6.06	3.12	10.34	3.45	5.90	-	-	-	-	-	9.09	-	-	-	-	6.25	-	7.69	6.45	-	-	-
A. flavus Link. ex. Fries.	-	-	-	10.34	-	-	4.76	-	-	-	-	-	16.64	-	4.16	-	-	-	-	3.44	-	11.76
A. niger Van Tieghem.	-	-	3.45	3.45	-	6.25	-	2.70	-	-	4.54	15.38	-	16.64	4.16	-	10.00	-	9.68	10.34	10.00	-
A. sydowi Thom. & Church.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.89	-	-
A. versicolor (Vuillemin) Tiraboschi.	9.09	3.12	10.34	-	11.76	-	-	-	-	-	13.60	-	-	-	-	-	-	-	-	-	10.00	5.88
Cephalosporium acremonium Corda.	-	6.30	3.45	6.89	11.76	-	-	-	-	-	-	-	33.30	-	-	-	5.00	15.38	-	-	6.70	-
Cladosporium herbarum Link. ex. Fries.	-	-	3.45	-	-	12.50	-	-	-	-	-	15.38	-	16.64	4.16	-	10.00	-	6.45	3.44	10.00	11.76
Fusarium solani	-	-	10.34	-	-	-	-	-	-	-	-	-	-	-	16.64	6.25	25.00	7.69	9.70	-	6.70	-
Fusarium sp.	-	-	-	-	11.76	12.50	-	-	-	37.50	-	-	-	-	-	-	-	-	-	-	-	11.76
Geotrichum sp.	-	6.30	6.89	10.34	-	-	19.04	13.50	8.30	-	-	-	-	-	8.33	6.25	5.00	15.38	6.45	10.34	10.00	-
Humicola sp.	-	-	-	6.89	-	-	-	-	-	-	4.54	15.38	-	-	8.33	-	-	7.69	-	3.44	-	5.88
Mucor hiemalis Wehmer.	-	3.12	-	-	-	12.50	9.52	10.80	-	12.50	9.09	-	16.64	-	-	-	-	-	9.68	-	-	5.88
Mucor sp.	9.09	-	-	-	5.90	6.25	4.76	-	-	-	-	7.69	-	-	8.33	6.25	10.00	7.69	-	10.34	-	-
Penicillium brefeldianum Dodge.	-	3.12	-	10.34	-	-	-	-	-	-	-	-	-	-		-	-	-	-	6.89	-	-
P. chrysogenum Thom.	12.12	-	13.79	10.34	-	18.80	9.52	21.60	-	12.50	22.70	23.00	-	-	-	6.25	-	-	9.68	-	6.70	11.76
P. granulatum Bainier.	-	3.10	-	-	-	-	-	-	16.70	-	-	-	16.64	-	-	-	-	-	-	-	-	-
P. nigricans Bainier.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.16	-	-	-	6.45	-	-	-
P. oxalicum Thom.	9.09	-	10.34	-	-	-	-	-	25.00	-	-	-	-	16.64	-	-	-	-	-	-	-	-
P. rubrum stoll.	9.09	25.00	10.34	-	17.66	6.25	-	27.00	-	-	4.54	7.69	-	-	-	-	-	-	6.45	-	6.70	-
P. vermiculatum Dangeard.	3.03	18.80	-	10.34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.70	-
Penicillium sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.22	-	-	5.88
Rhizopus stolonifer (Ehrenb ex.Link) Lind 1913.	15.20	-	13.79	10.34	5.90	12.50	-	10.80	-	12.50	9.09	15.38	-	33.30	20.82	31.25	15.00	7.69	16.13	6.89	13.30	11.76
Trichoderma aureoviride Rifai.	-	-	-	-	-	6.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. harzianum Rifai.	-	-	-	-	-	-	9.52	8.10	16.70	-	9.09	-	-	-	-	-	-	-	-	-	-	-
T. koningii oudemans.	-	-	3.45	3.45	-	6.25	-	-	-	-	-	-	-	-	8.33	-	-	-	-	-	-	5.88
T. viride Pers. ex. Fries.	3.03	-	-	-	17.66	-	-	-	-	12.50	-	-	16.64	-	-	-	-	-	-	-	-	-
Verticillium sp.	3.03	-	-	-	-	-	-	-	-	-	-	-	-	16.64	-	-	-	-	-	-	-	-
Yeast (red)	15.20	18.80	-	6.89	11.76	-	23.80	5.40	8.30	-	13.63	-	-	-	8.33	18.75	10.00	23.07	6.45	31.63	13.30	11.76
Unidentified	-	9.37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Antibiotics sprayed to soybean plants at a rate of 20, 40, 80, 100 ug/L.

TABLE 5.6 : Effect of antibiotics on the disease development of soybean plant.

Antibiotics	Concentration (µg/L)	Percentage of infection/weeks[1]									
		5	6	7	8	9	10	11	12	13	14
Actidione	20	-	6.7	6.7	13.3	16.7	26.7	26.7	33.3	33.3	40.0
	40	-	-	3.3	6.7	6.7	13.3	23.3	26.7	33.3	33.3
	80	-	-	-	3.3	6.7	10.0	16.7	16.7	23.3	26.0
	100	-	-	-	3.3	6.7	6.7	13.3	16.7	16.7	20.0
Streptomycin	20	6.7	10.0	10.0	16.7	20.0	26.7	33.3	43.3	46.7	56.7
	40	3.3	6.7	6.7	13.3	20.0	23.3	30.0	40.0	43.3	53.3
	80	-	6.7	10.0	10.0	16.7	20.0	23.3	30.0	33.3	40.0
	100	-	-	3.3	6.7	10.0	10.0	20.0	26.7	30.0	33.3
Chloramphenicol	20	-	6.7	13.3	16.7	16.7	23.3	26.7	36.7	46.7	46.7
	40	-	3.3	6.7	13.3	13.3	20.0	26.7	26.7	33.3	36.7
	80	-	-	3.3	10.0	13.3	13.3	16.7	23.3	26.7	30.0
	100	-	-	-	6.7	10.0	16.7	16.7	20.0	23.3	30.0
Penicillin	20	3.3	3.3	10.0	16.7	20.0	26.7	30.0	40.0	46.7	66.0
	40	-	3.3	6.7	6.7	13.3	20.0	23.3	26.7	26.7	36.7
	80	-	-	-	6.7	13.3	16.7	23.3	26.7	33.3	33.3
	100	-	-	-	3.3	3.3	6.7	10.0	16.7	16.7	23.3
Thiolutin	20	-	3.3	10.0	16.7	20.0	26.7	36.7	46.7	50.0	50.0
	40	-	-	3.3	6.7	10.0	16.7	20.0	26.7	30.0	40.0
	80	-	-	3.3	6.7	6.7	13.0	16.7	26.7	30.0	36.7
	100	-	-	-	3.3	10.0	10.0	13.3	16.7	23.3	26.7
Control (without treatment)	-	16.7	26.7	30.0	40.0	43.3	50.0	56.7	66.7	70.0	76.7

1) Calculation based on 30 plants in each case.

Plant height

Decreased height in most of the treated plant was observed excepting lower concentration (20, 40 µg/L) of streptomycin and thiolutin (Plate 15) compared to non sprayed infected control. Highest reduction was observed with penicillin treated plants. It was also observed that higher concentration of all the antibiotics were toxic to plant as it directly inhibited the growth (Table 5.7).

Yield

Increased yield was recorded with most of the antibiotics excepting actidione (all the concentrations tested), chloramphenicol (80 and 100 µg/L), penicillin and thiolutin (100 µg/L). Thiolutin, chloramphenicol (lower concentration) followed by streptomycin gave comparatively better yield (Fig. 7.1).

DISCUSSION

The control of diseases might have occurred either by an inhibitory effect of the chemicals on the pathogen or by inducing disease resistance in the treated plants. All the antibiotics tested in the present study were found to reduce the disease severity of soybean. Most of the antibiotic sprayed soybean plant showed a delay in symptom expression. Davis and Dimond (1952) postulated that external application

Plate 15: Effect of foliar spray of (i) Streptomycin and (ii) Thiolutin on the disease control and increased plant height compared to infected control.

PLATE-15


INFT. CONTROL
20 µg/L
40 µg/L

(i)


INFT. CONTROL
THIOLUTIN
20 µg/L
40 µg/L
80 µg/L

(ii)

TABLE 5.7 : Effect of foliar spray of antibiotics on the growth[1] (plant height in cm) of soybean plant.

Antibiotics	Concentration (µg/L)	Age in weeks					
		5	6	7	8	9	10
Actidione	20	18.84±0.59	27.50±0.86	34.00±1.06	41.00±1.09	49.90±1.02	54.70±0.57
	40	18.21±0.52	25.70±0.47	29.80±0.82	34.10±0.78	42.90±1.19	47.70±0.67
	80	18.00±0.49	23.70±0.98	27.00±1.59	31.00±0.82	40.90±1.89	45.90±0.08
	100	18.80±0.57	20.50±0.70	26.00±1.30	31.40±1.73	38.30±1.71	42.90±0.86
Streptomycin	20	18.75±0.60	26.40±0.91	30.15±0.90	38.78±1.37	47.60±1.44	56.60±1.09
	40	18.77±0.50	20.60±1.04	29.00±2.02	35.50±2.48	42.40±1.10	54.00±1.68
	80	17.64±0.45	19.30±1.24	25.00±1.46	30.58±1.59	40.20±2.04	49.50±1.29
	100	16.17±0.49	18.00±0.51	24.18±1.19	30.00±1.03	36.00±1.13	45.00±0.83
Chloramphenicol	20	18.02±0.61	23.70±1.07	29.30±2.02	34.60±1.06	39.87±1.04	48.70±1.44
	40	17.40±0.46	24.06±0.89	28.00±1.37	35.40±2.13	40.60±1.13	46.60±2.94
	80	16.80±0.35	20.15±0.45	26.67±1.53	33.00±1.03	37.25±1.67	42.00±2.10
	100	17.10±0.76	18.82±0.67	24.58±1.41	31.40±1.73	33.60±1.25	39.92±1.04
Penicillin	20	22.30±0.45	28.70±1.45	33.00±2.08	38.50±1.39	42.56±1.03	45.30±1.20
	40	20.00±0.70	24.40±0.67	28.40±0.89	31.00±0.82	38.40±1.50	44.20±1.55
	80	18.94±0.69	23.00±0.41	27.70±1.36	34.80±1.90	40.00±1.42	43.70±1.19
	100	17.60±0.66	21.25±0.52	26.18±1.69	31.00±1.22	34.70±1.59	39.90±1.29
Thiolutin	20	20.15±0.52	24.00±0.59	30.36±0.98	39.75±1.52	46.30±1.20	54.30±2.29
	40	19.70±0.43	22.01±0.55	28.50±0.53	35.30±1.42	44.30±1.20	52.30±1.19
	80	18.30±0.59	20.60±0.36	24.58±1.33	32.50±1.54	40.60±1.48	47.60±1.92
	100	17.60±0.28	19.64±0.59	23.30±1.37	30.36±1.23	37.36±1.50	41.40±1.22
Infected control (without treatment)	-	22.00±0.43	26.40±1.53	37.27±1.89	40.85±1.29	44.67±1.08	49.87±1.64

1) Mean of 10 plants with ±S.E.

Fig. 7.1: Effect of antibiotic foliar spray on the yield produced by soybean plants grown in **Sclerotium rolfsii** infested soil.

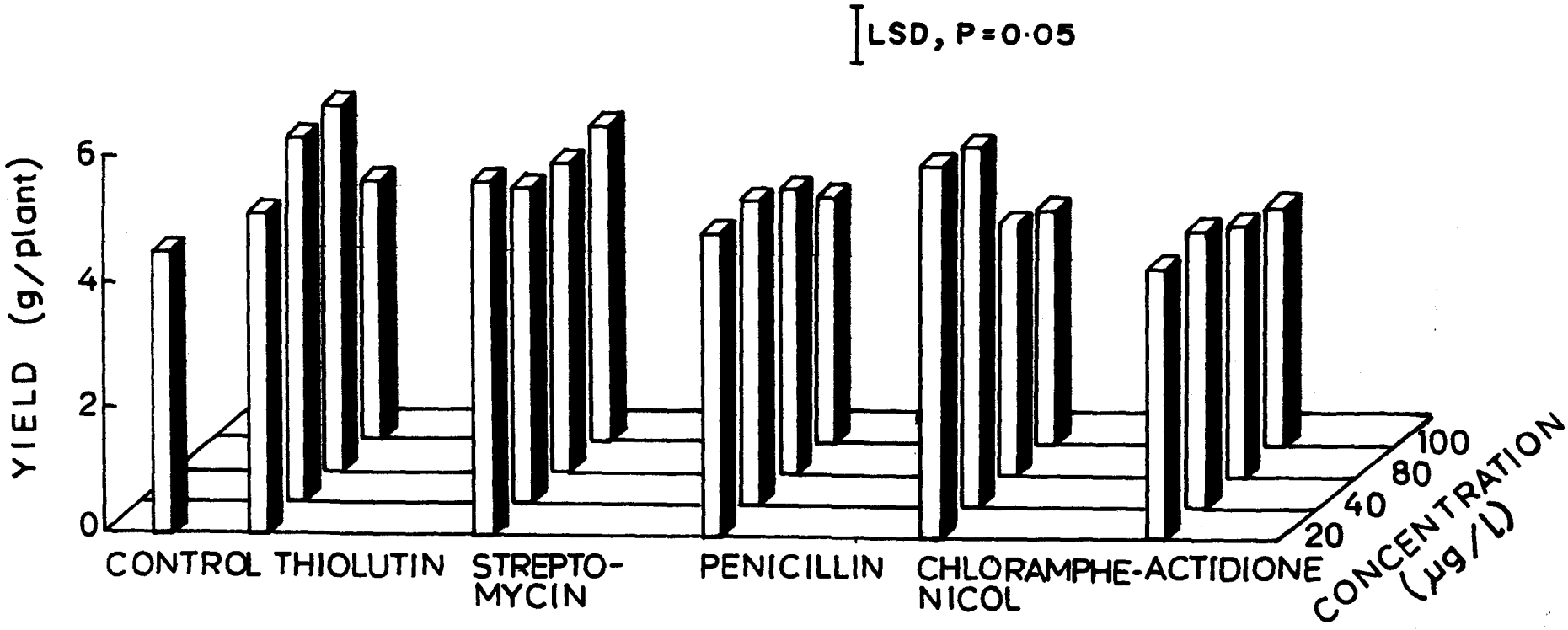

LSD, P=0·05
YIELD (g/plant)
6
4
2
0
CONTROL
THIOLUTIN
STREPTO-MYCIN
PENICILLIN
CHLORAMPHE-NICOL
ACTIDIONE
20
40
80
100
CONCENTRATION (µg/l)

FIG.7·1

of some chemicals (i.e. growth regulators) increased resistance to disease by inducing changes in the host metabolic process. Disease control by the antibiotics having less fungitoxic effect (e.g, chloramphenicol, streptomycin, penicillin) seemed to be due to a change in host metabolism, together with changed rhizosphere microflora, resulting in a reduction in the disease severity.

Among the antibiotics tested, actidione and thiolutin showed toxic effect to **S. rolfsii** by completely inhibiting the growth even at lower concentration (i.e. 20 µg/L). Reduction in the viability of sclerotium (50%) after 15 days of incubation in the antibiotic treated soil was observed in most of the cases. Thiolutin completely reduced the viable sclerotia within 60 days in soil, suggesting its fungitoxic nature. Similar results with thiolutin was also reported by Gopalkrishnan and Jump (1951), where they obtained complete inhibition in growth of **Pythium debaryanum, Sclerotinia fructicola** at 10 ppm and **Rhizoctonia solani** and **Colletotrichum gossypii** at 50 ppm. Dutta and Isaac (1981) also have reported that thiolutin and actidione were found to be very active against **Verticillium spp. in vitro.**

Antibiotic treatment drastically reduced the fungal and bacterial population in the soybean rhizosphere, whereas actinomycete found to be stimulated. Similar results were also reported earlier with thiolutin (Deb and Dutta, 1984).

A reduction in bacterial population as a result of chloramphenicol treatment was also reported by Vrany **et al** (1962). It is known that chloramphenicol is active against a number of bacteria and interfere in the protein synthesis (Newton, 1965), consequently a reduction in bacterial population occurred, Baruah and Dutta (1978) also reported that the antibiotic foliar spray had profound effect on the distribution of the microflora as well as on the percentage occurrence of certain soil fungi in the rhizosphere of tomato plants infected with **Verticillium albo-atrum**. They further observed that streptomycin and actidione reduced the fungal population, while chloramphenicol reduced the bacterial and actinomycete population in the tomato rhizosphere, which is similar to the results obtained in the present work. The absence of direct evidence on the effect of antibiotics on the physiology of the plants, it is assumed that the changed rhizosphere effect and the effect on the particular genera of fungi is due to the possible change in the root exudate pattern caused by the foliar spray of antibiotics.

Antibiotics (i.e. Polyoxins) are known to interfere with cell wall chitin synthesis by competitive inhibition of the enzyme, chitin synthetase (Hori **et al**, 1971, 1974). Actidione and streptomycin inhibit protein synthesis (Whiffen, 1948; Hahn **et al**, 1962) while some antibiotic (i.e. griseofulvin) seems to interfere with mitosis (Gull and Trinci, 1974),

interfere with synthesis of nucleotide or replication of DNA (i.e. thiolutin) (Dekker, 1976). In the present experiment thiolutin and actidione was found to be fungitoxic **in vitro** while others were non-fungitoxic. To interpret the exact mechanism by which the antibiotics used in the present experiment changed the pathogen/host physiology and decreased the disease severity, requires further experimentation.

Toxic nature of actidione, chloramphenicol and thiolutin (higher concentration) to soybean directly affected the soybean yield. Streptomycin and lower concentration of thiolutin and chloramphenicol produced better yield, together with the decrease in disease severity suggests that these concentrations are suitable for control of the disease caused by **S. rolfsii** and can be used as chemotherapeutic agent. Results of the present investigation suggests that further screening of large number of antibiotics against **S. rolfsii** be needed, which can be utilised for the control of diseases caused by **S. rolfsii.**

CHAPTER VI

EFFECT OF FUNGICIDES (AS SOIL DRENCH) ON THE RHIZOSPHERE MICRO-FLORA, DISEASE DEVELOPMENT, PLANT VIGOUR AND YIELD IN SOYBEAN PLANTS GROWN IN SCLEROTIUM ROLFSII INFESTED SOIL.

INTRODUCTION

Before the nineteenth century the main work of the mycologist was naming and classifying fungi according to Linnaean system. This involved detailed observation of the organisms concerned. To some botanist the disease structure constantly associated with a specific symptoms, was due to the presence of the fungus and not merely due to host plant as it had been thought previously. However, at this time also, some confusion still existed on the true nature of plant diseases. Subsequently, the control of the disease development in host plants caused by microorganisms also gained momentum. Most of the early attempts of chemical control were based on natural products of one kind or another (i.e. vegetable oils). Until about 1930, the dominant chemicals used were either sulphure or copper sulphate in combination with lime. Several organo-metallic compounds were also used for the control of plant diseases (Martin, 1940). Subsequently, treatment of soil with chemicals and the use of lime/sulphur mixture was introduced for the disease control. Mc.Cllan (1930) described the design of technique aimed at selecting the concentration for better fungicides without affecting the host plant and this have since formed the basis of the modern disease control techniques. Progress with chemical control of soil borne disease can only be efficiently planned if the biological nature of the problem is adequately understood

and therefore, was given due consideration.

Fungicides are known to kill or inhibit the growth and development of fungal spores and mycelium. They are either of protectant, systemic or eradicant in nature, on the basis of their uptake and mobility within the plant.

Protectant fungicides usually applied to seeds, soil or on the plant surface to prevent infection by the pathogen. These fungicides cannot penetrate into plant tissue in effective amount. By contrast, systemic fungicides usually are taken up and translocated throughout the plants, thereby cured an established infection.

In the present work an observation was made to determine the efficacy of some fungicides (i.e. agallol, PCNB, agrosan, sulfex, ziride, dicloran, delan) against the disease development in soybean plants caused by **S. rolfsii**. Their effect in relation to the change in rhizosphere microflora, plant vigour and yield was also taken into consideration.

MATERIALS AND METHODS

After the screening of several fungicides against the growth of **S. rolfsii, in vitro,** seven promising fungicides were selected for phytopathological experiments. The commercially available wettable powder of the fungicides mentioned below were used in aqueous solution in the present study.

Common name or Trade name	Wettable powder	Chemical compound
1. Agallol	3%, 6%	Methyl ethyl mercury chloride.
2. PCNB, Quintozone, Brassicol, Terraclor.	75 WP	Pentachloro nitrobenzene.
3. Agrosan G.M.	1%	Phenyl mercury acetate+ ethyl mercury chloride.
4. Sulfex	80 WP	Elemental sulphur.
5. Ziride or ziram	80 WP	Zinc dimethyl dithiocarbamate.
6. Dicloran, Botran, DCNA	-	2,6 Dichloro-4-nitro aniline.
7. Delan	75% WP	Dithianon (2,3,dicarbanitrile).

A stock suspension of each fungicides was prepared by extracting a known amount of wettable powder in 1ml acetone and then 99ml of sterile water was added. An equal amount of acetone was also added to the control set without any fungicide. The amount of acetone never exceeded 2%, which was found to be the amount having virtually no fungal toxicity or phytotoxic effect.

Different concentrations (i.e. 20, 40, 80, 100 µg/L) of the fungicides prepared thus, were tested against the growth, survibality of the sclerotium of **S. rolfsii**, soybean seed germination, phytotoxicity and on the rhizosphere microflora of soybean in relation to the disease development.

Soybean seedlings were raised (10 plants/pot) in

S. rolfsii infested natural soil (at ICAR farm, Barapani) and when they were one month old, fungicides at different concentrations were drenched thoroughly in the soil around the collar region. Tap water was drenched to the soil containing soybean plants, which serves as control. Four replicates were maintained for each treatment.

After 15 days of soil drench, ten seedlings from each treatment were uprooted gently without disturbing the root system along with adhering soil. Rhizosphere population and 'rhizosphere effect' were determined as described earlier (page 39). Percentage of relative abundance of particular genera/species of fungi was also recorded.

Disease development/severity, seedlings growth and yield of soybean plant was also recorded as described in page 42 .

RESULTS

Among the fungicides tested, agrosan and PCNB showed highest inhibition in growth of **S. rolfsii in vitro.** Others also showed varied inhibitory effect (Fig. 8.1). The inhibition increased with the increase in the concentration of the fungicides tested. 50-66% inhibition in the growth of **S. rolfsii** was recorded with all the fungicides tested here, at 80 and 100 µg/L concentration.

Fig. 8.1: Effect of fungicides on the radial growth of **Sclerotium rolfsii in vitro.**

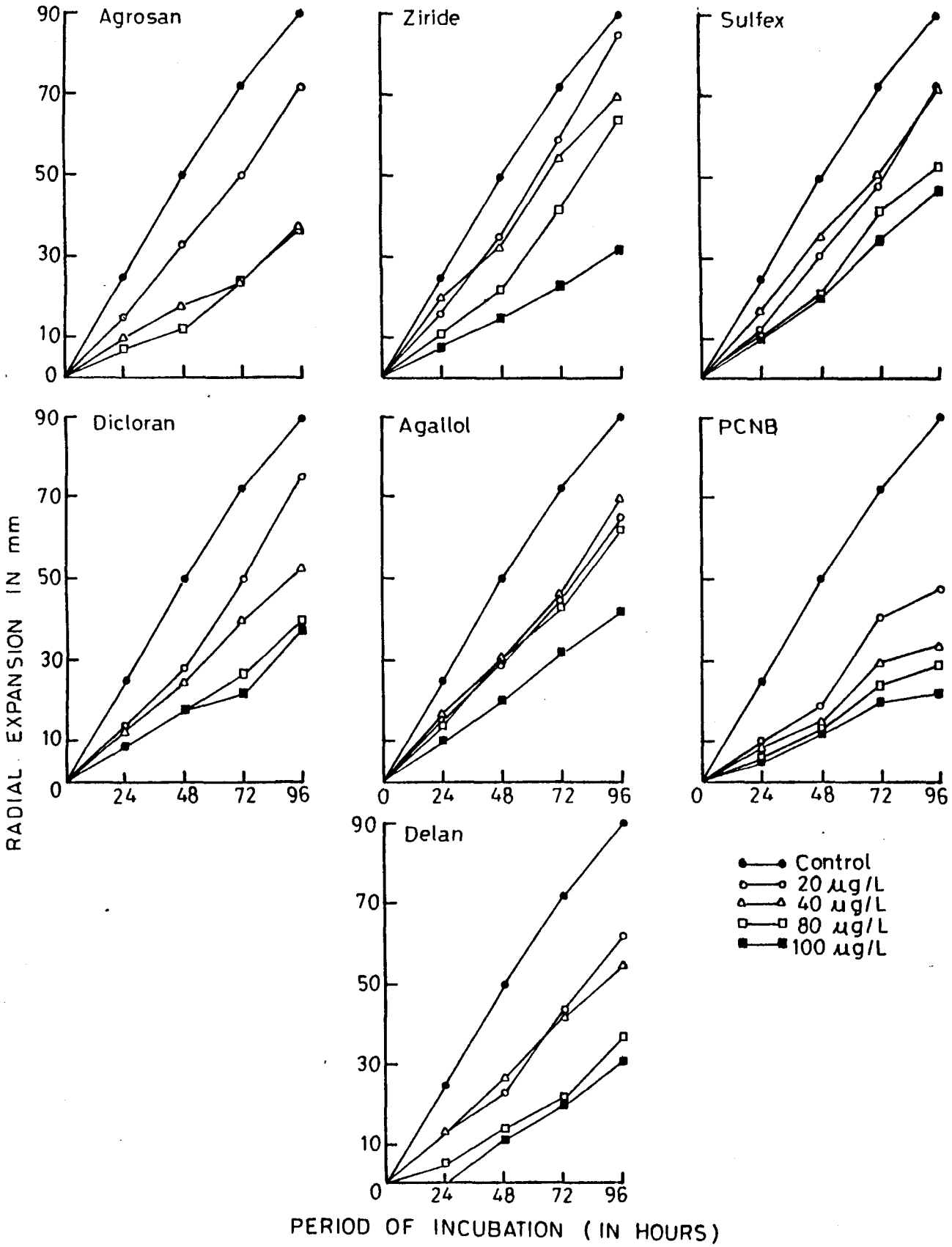

Agrosan
Ziride
Sulfex
Dicloran
Agallol
PCNB
Delan
RADIAL EXPANSION IN mm
PERIOD OF INCUBATION (IN HOURS)
0
10
30
50
70
90
24
48
72
96
Control
20 µg/L
40 µg/L
80 µg/L
100 µg/L

Fig.8·1

The fungicides were found to be toxic in nature, reducing the germination (%) of the sclerotium in aqueous solution as well as in soil. The active fungicides were found to be sulfex and agrosan. Higher concentration of these fungicides reduced the germination of sclerotium as observed after seven days of incubation in aqueous solution. In soil, the number of viable sclerotia reduced after 15 days of incubation (i.e. at higher concentration of most of the fungicides). The viability decreased with the increase in incubation period in the soil. Dicloran and agallol were found to be the most effective fungicides, which reduced the survibality of sclerotia in the soil (Table 6.1).

Phytotoxicity

Soybean seed germination was not affected **in vitro** excepting at higher concentration of sulfex, dicloran and PCNB. When the seeds were soaked with the aqueous solution of fungicides, radicle growth was found to be slightly inhibited, in most of the cases. Root dip treatment of seedlings showed no toxic effect, excepting higher doses of sulfex (80, 100 µg/L) where yellowing of leaves was observed (Table 6.2).

Effect on the rhizosphere

Reduced microbial population was observed in the rhizosphere of the fungicide treated plants. Fungal population

TABLE 6.1 : Effect of fungicides on the survibality of Sclerotium.

Fungicides	Concentration (µg/L)	Sclerotium Germination(%)				
		In aqueous solution after 7 days[1]	In soil[2] (Period of Incubation (Days))			
			15	30	45	60
Agrosan	20	100.00	100.00	90.90	85.70	70.00
	40	86.60	100.00	80.00	70.00	70.00
	80	46.60	100.00	76.90	66.70	55.00
	100	33.30	100.00	60.00	50.00	50.00
Ziride	20	100.00	100.00	70.00	60.00	55.50
	40	100.00	76.90	66.70	56.30	54.50
	80	93.30	60.00	50.00	46.70	37.50
	100	86.60	56.30	46.70	25.00	21.40
Sulfex	20	53.30	90.00	76.90	50.00	46.15
	40	33.30	60.00	56.25	45.50	33.30
	80	26.70	50.00	37.50	31.25	26.70
	100	13.30	50.00	30.00	27.00	22.20
Dicloran	20	100.00	73.30	62.50	37.50	33.30
	40	66.70	70.00	56.30	40.00	35.00
	80	73.30	62.50	50.00	33.30	16.00
	100	53.30	33.30	33.30	20.00	12.50
Agallol	20	66.70	100.00	81.80	70.60	50.00
	40	60.00	100.00	60.00	50.00	50.00
	80	53.30	70.00	50.00	40.00	33.30
	100	53.30	40.00	30.80	28.60	14.30
PCNB	20	93.30	100.00	85.70	80.00	80.00
	40	93.30	100.00	86.70	73.30	84.20
	80	80.00	91.70	70.00	76.90	83.40
	100	60.00	75.00	66.70	73.30	70.00
Delan	20	93.30	100.00	80.00	66.70	46.15
	40	86.70	66.70	50.00	38.46	33.30
	80	86.70	62.50	41.70	27.80	21.40
	100	80.00	50.00	37.50	25.00	18.75
Control (without treament)	-	100.00	100.00	100.00	100.00	90.00

1) Calculation based on 60 sclerotia in each case.
2) Calculation based on recovered sclerotia.

TABLE 6.2 : Effect of fungicides on Soybean seed germination and radicle growth.

Fungicides	Concentration (µg/l)	Seed germination (%)[1]	Radicle length (cm)[2]	Phyto-toxicity
Agrosan	20	100.00	13.10±1.44	-
	40	100.00	12.20±0.94	-
	80	10 0.00	10.10±1.03	-
	100	100.00	9.40±1.12	-
Ziride	20	100.00	10.00±1.54	-
	40	100.00	8.00±1.34	-
	80	100.00	9.00±1.00	-
	100	100.00	9.28±1.06	-
Sulfex	20	100.00	11.40±0.82	-
	40	100.00	11.00±1.64	-
	80	93.30	10.70±1.87	+
	100	80.00	7.16±0.79	+
Dicloran	20	100.00	13.30±0.88	-
	40	100.00	13.16±1.08	-
	80	100.00	12.30±1.60	-
	100	86.70	9.00±0.71	-
Agallol	20	100.00	11.90±0.97	-
	40	100.00	11.00±1.00	-
	80	100.00	9.40±0.70	-
	100	100.00	9.30±0.59	-
PCNB	20	100.00	11.80±1.14	-
	40	100.00	11.70±1.28	-
	80	93.30	9.70±1.08	-
	100	93.30	9.70±0.99	-
Delan	20	100.00	13.43±1.13	-
	40	100.00	11.00±1.09	-
	80	100.00	10.85±0.88	-
	100	100.00	9.38±1.00	-
Control (without treatment)	-	100.00	12.00±1.25	-

1) Calculation based on 30 seeds in each case.
2) Mean of 10 seedlings with ±S.E.; (-) Nil; (+) Slightly toxic.

decreased to a great extent following soil drench with sulfex and ziride, whereas, slight increase in population was also observed due to agallol (at 20, 40 µg/L) treatment. Actinomycete and bacterial population found to reduce significantly. Treatment with delan however, showed a slight increase in the bacterial population in the soybean rhizosphere (Table 6.3).

In general, the application of fungicides increased the population of Aspergilli considerably in the rhizosphere of soybean, while in some cases i.e. delan (all the concentration), ziride (20, 40, µg/L), sulfex (40, 80, 100 µg/L) this population found to be reduced. Mucorales population also increased in the rhizosphere following ziride (all the concentration), sulfex (20, 80, 100 µg/L) and delan (40, 80 µg/L) treatment. **Penicillium** spp. population decreased excepting sulfex (40 µg/L), dicloran (40 µg/L), agallol(80 µg/L) and delan (lower concentration only) treatment. Some other genera of fungi were found to be slightly boosted. These were **Cephalosporium** spp., **Cladosporium** spp. and **Trichoderma** spp. **Fusarium** spp. also increased in rhizosphere following agrosan (20, 40 µg/L), dicloran (40, 80, 100 µg/L) and agallol (40, 80 µg/L) treatment (Table 6.4).

Altogether, thirty species belonging to fourteen genera have been isolated from the rhizosphere of soybean. The percentage of relative abundance of the same have been

TABLE 6.3 : Effect of soil drench of fungicides on the rhizosphere microflora of soybean grown in pathogen infested soil.

Soil amendments	Concentration (µg/L)	Rhizosphere micro-organisms in thousands g^{-1} dry soil[1]			Rhizosphere effect (RS/CS)[2]		
		Fungi (10^4)	Actinomycete ($2x10^4$)	Bacteria ($3x10^4$)	Fungi	Actinomycete	Bacteria
Agrosan	20	14.58	91.27	206.75	1.45	0.28	0.91
	40	16.27	86.27	195.00	1.62	0.26	0.86
	80	17.08	83.77	154.16	1.70	0.25	0.68
	100	18.77	82.50	126.70	1.87	0.25	0.56
Ziride	20	15.00	154.17	170.80	1.50	0.47	0.75
	40	11.27	135.40	156.70	1.13	0.41	0.69
	80	9.16	132.00	152.00	0.92	0.40	0.67
	100	9.16	119.58	141.60	0.92	0.36	0.62
Sulfex	20	8.77	185.56	204.00	0.88	0.56	0.90
	40	7.10	179.58	162.50	0.70	0.54	0.72
	80	7.53	216.70	151.70	0.75	0.66	0.67
	100	7.27	226.40	99.58	0.70	0.69	0.44
Dicloran	20	17.10	150.00	288.80	1.70	0.45	1.00
	40	15.40	182.00	219.00	1.54	0.55	0.97
	80	11.25	218.80	130.80	1.12	0.66	0.58
	100	12.20	222.90	117.90	1.08	0.67	0.52
Agallol	20	35.00	183.30	212.50	3.50	0.55	0.94
	40	32.00	178.40	166.70	3.20	0.47	0.74
	80	25.80	131.70	121.70	2.58	0.40	0.54
	100	18.30	95.40	120.00	1.83	0.29	0.53
PCNB	20	19.80	194.33	139.60	1.98	0.59	0.62
	40	17.00	185.40	204.00	1.70	0.56	0.90
	80	15.90	138.40	200.80	1.58	0.42	0.89
	100	10.40	120.80	226.70	1.04	0.37	1.00
Delan	20	13.40	190.80	330.80	1.33	0.58	1.46
	40	12.90	176.70	262.50	1.29	0.53	1.16
	80	12.10	169.50	208.90	1.58	0.51	0.92
	100	14.16	141.25	141.70	1.42	0.43	0.63
Control rhizosphere soil	-	30.00	347.90	275.00	3.00	1.05	1.21
Control soil	-	10.00	330.80	226.70	-	-	-
Significance by F test (1%)	-	Yes	Yes	Yes			

1) Mean of three replicates in each case.
2) Rhizosphere soil/control soil.

TABLE 6.4 : Effect of soil drench of fungicides on the percentage of relative abundance of major groups of fungi in soybean rhizosphere in pathogen infested soil.

Fungicides	Concentration (µg/L)	Aspergilli	Cephalosporium spp.	Cladosporium spp.	Fusarium spp.	Geotrichum spp.	Mucorales	Penicillia	Yeast	Trichoderma spp.	Other genera of fungi
Agrosan	20	49.99	-	-	14.28	-	7.14	11.90	7.14	9.52	-
	40	53.10	-	-	19.60	2.80	-	-	11.10	8.23	5.60
	80	22.60	6.50	-	12.90	-	9.67	9.67	22.60	9.67	6.50
	100	37.88	-	-	3.44	13.80	17.24	6.89	17.24	-	3.44
Ziride	20	13.63	-	-	-	-	45.45	22.72	9.09	-	0.09
	40	5.60	-	-	5.60	11.20	39.20	-	16.70	11.20	11.20
	80	31.79	-	4.54	-	9.09	40.87	-	4.54	-	9.09
	100	36.35	9.09	-	-	-	31.81	-	22.70	-	-
Sulfex	20	23.78	-	4.76	-	-	28.54	4.76	28.57	4.76	4.76
	40	17.66	-	-	11.76	-	17.66	29.44	11.76	-	11.76
	80	-	5.60	-	-	27.80	39.00	-	11.20	5.60	11.20
	100	11.76	5.90	11.76	-	-	35.30	17.66	11.76	5.90	-
Dicloran	20	44.00	4.00	8.00	4.00	-	24.00	4.00	8.00	4.00	-
	40	20.00	-	6.70	13.30	-	6.70	23.30	20.00	-	10.00
	80	23.03	-	-	3.80	7.70	19.23	7.70	19.23	19.23	-
	100	22.20	-	7.40	18.52	-	3.70	18.52	18.52	7.40	3.70
Agallol	20	32.72	10.90	-	7.27	-	14.54	20.00	-	1.09	12.70
	40	41.75	1.49	-	19.40	5.97	10.40	5.97	11.97	1.49	1.49
	80	22.70	-	6.81	25.00	6.81	6.81	25.00	-	6.81	-
	100	32.25	4.83	1.60	12.90	1.61	3.22	2.96	9.68	3.22	9.66
PCNB	20	20.00	4.00	-	8.00	4.00	16.00	16.00	16.00	16.00	-
	40	30.00	3.30	10.00	3.30	-	13.30	13.30	10.00	16.70	-
	80	34.60	-	15.38	7.69	-	11.53	11.53	3.84	7.69	7.69
	100	22.53	-	16.13	-	3.22	22.58	16.12	12.90	3.22	3.22
Delan	20	15.63	9.37	6.25	6.25	9.37	18.76	28.11	6.25	-	-
	40	12.90	6.45	3.22	-	-	32.25	25.80	12.90	6.45	-
	80	13.04	4.34	4.34	4.34	-	34.77	17.39	13.04	4.34	4.34
	100	12.00	8.00	-	8.00	8.00	12:00	12.00	16.00	8.00	16.00
Control rhizosphere soil	-	21.00	-	-	12.50	12.50	20.90	21.00	-	-	12.50
Control soil	-	25.00	-	6.25	12.50	-	12.50	18.75	6.25	6.25	12.50

presented in the table 6.5.

Effect on the disease development

In the initial stage, all the 5 weeks old soybean plant, irrespective of fungicide treatment (excepting agrosan and PCNB, 100 µg/L) showed varied disease symptom/severity. The percentage of infection was much less in the treated plants compared to the non treated infected control. PCNB was found to be the most effective among the fungicides tested (Fig. 8.2). Higher concentration (100 µg/L) of all the fungicides used in the present study, showed approximately 50% reduction in disease severity compared to infected control. PCNB and agrosan, delayed the symptom expression by one week following the soil drench.

Effect on plant height

A considerable increase in plant height was recorded at the initial stage of growth but later (i.e. at the age of seventh week) the growth of the plants was found to be slower. Slight increase in plant height and improved vigour was observed in case of PCNB, agrosan and dicloran only (Table 6.6).

Effect on yield

Highest yield of soybean was recorded in the plant which received PCNB and agrosan as soil drench. Lower concentration

Fig.8.2: Effect of fungicides soil drench on the disease development of soybean grown in **Sclerotium rolfsii** infested soil.

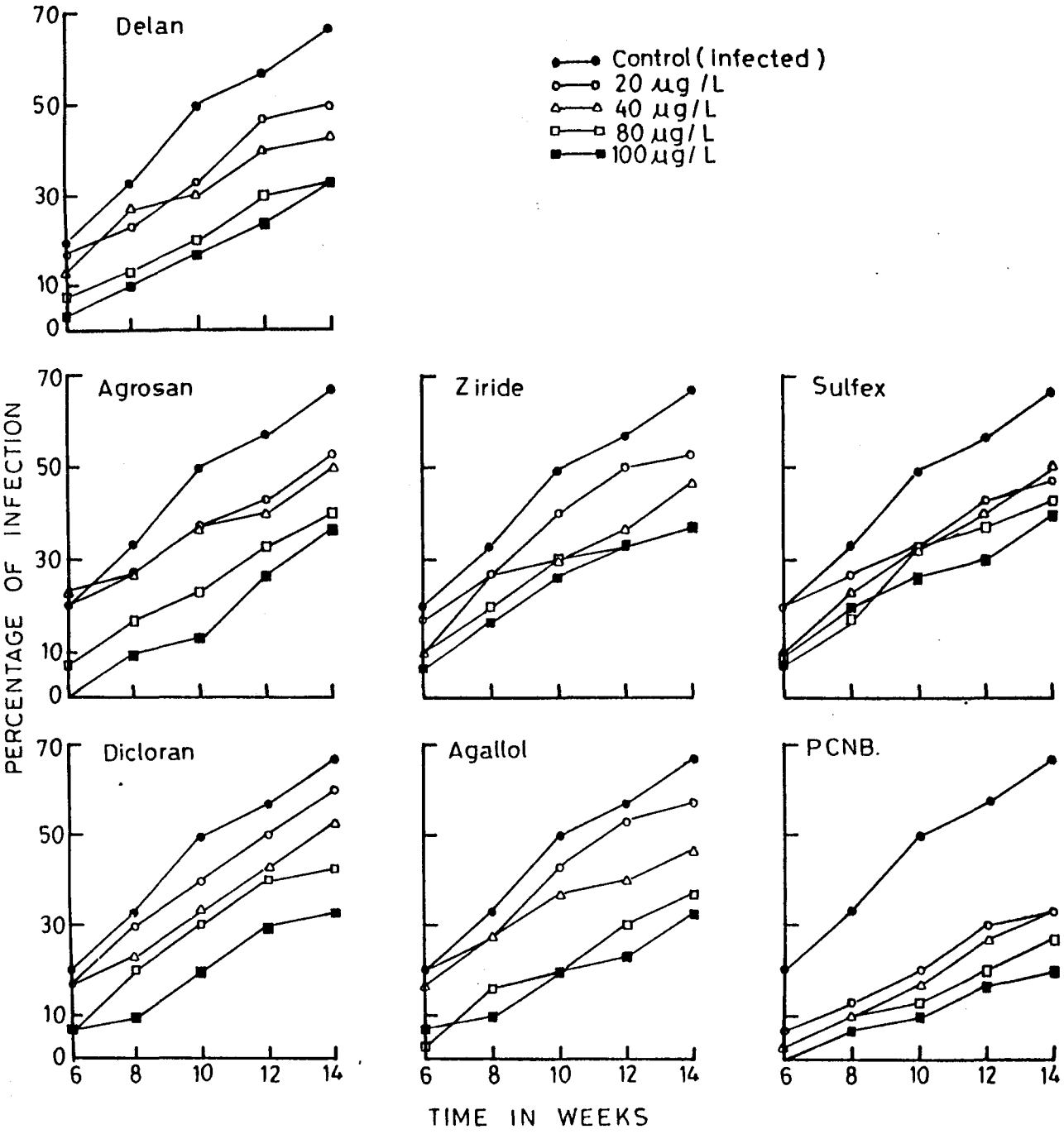

Delan
Agrosan
Ziride
Sulfex
Dicloran
Agallol
PCNB.
Control (Infected)
20 µg /L
40 µg/L
80 µg/L
100 µg/L
PERCENTAGE OF INFECTION
TIME IN WEEKS
0
10
30
50
70
6
8
10
12
14

Fig. 8·2

TABLE 6.6 : Effect of fungicides on the growth[1] (plant height in cm) of soybean plant.

Fungicides	Concentration (µg/L)	Age in weeks					
		5	6	7	8	9	10
Agrosan	20	27.90±1.29	32.70±0.99	34.60±0.78	43.67±1.03	48.45±1.44	51.90±0.68
	40	26.60±1.59	30.70±0.90	35.30±0.69	42.80±1.04	46.50±2.94	49.60±0.67
	80	27.80±1.47	31.00±1.03	37.30±1.14	39.60±2.73	45.70±1.24	49.10±0.55
	100	24.50±1.19	30.00±0.98	38.38±1.67	40.40±1.52	45.30±1.36	45.60±0.83
Ziride	20	21.20±0.64	27.00±0.79	34.60±1.03	40.75±1.86	42.60±2.59	49.54±0.58
	40	16.80±0.83	19.60±1.22	30.20±1.22	37.90±1.71	40.80±2.04	46.60±0.59
	80	18.50±0.82	28.54±1.12	37.00±1.48	40.50±1.09	42.00±1.14	47.40±0.85
	100	17.80±0.66	23.40±0.78	31.50±1.73	39.00±1.39	42.70±0.86	42.80±0.56
Sulfex	20	21.60±0.96	24.40±1.41	32.30±2.03	37.67±1.13	41.27±1.43	46.36±0.81
	40	19.70±0.52	22.60±1.03	32.10±0.69	37.27±1.82	39.60±1.04	43.50±0.82
	80	20.20±0.76	22.10±0.81	29.90±1.53	35.70±0.83	36.00±1.25	42.50±0.60
	100	19.20±0.87	22.20±1.32	29.80±0.99	34.70±0.97	36.30±1.61	40.00±0.53
Dicloran	20	25.30±1.33	31.90±0.53	39.00±1.39	45.10±1.42	50.20±1.10	54.60±0.57
	40	25.50±1.30	29.80±0.95	35.60±0.90	39.70±0.97	42.40±2.10	50.60±0.87
	80	26.90±0.96	28.70±0.56	37.04±1.10	47.50±1.19	49.55±1.65	51.30±0.75
	100	24.80±1.53	27.20±1.59	34.60±1.42	42.40±6.37	46.40±1.29	49.10±0.82
Agallol	20	19.00±1.01	24.30±0.89	31.90±0.92	38.62±1.89	43.50±1.29	46.30±0.88
	40	23.00±1.25	25.00±1.36	29.00±2.02	35.22±2.48	39.66±1.53	42.75±0.56
	80	19.75±1.43	21.90±1.17	26.10±1.69	31.60±0.71	38.10±1.50	41.10±0.63
	100	19.03±1.00	21.40±1.13	25.10±1.76	33.40±1.59	36.90±0.86	40.70±0.61
PCNB	20	29.10±1.19	34.90±1.06	41.70±1.57	45.30±1.20	46.40±2.30	52.40±0.58
	40	25.80±1.48	29.00±2.02	35.25±1.46	40.50±1.89	45.56±1.30	54.54±0.58
	80	26.90±1.04	30.60±0.82	38.20±1.86	42.50±1.19	43.60±1.02	49.80±0.51
	100	23.80±1.42	30.40±1.53	34.40±1.58	43.09±2.48	49.60±1.13	52.30±0.55
Delan	20	23.58±1.58	26.80±1.65	31.00±0.82	38.00±1.29	45.00±1.08	51.44±0.71
	40	23.40±0.78	26.70±1.33	30.20±0.91	41.00±0.99	43.00±1.10	47.20±0.67
	80	19.20±1.24	24.80±1.46	29.60±0.89	37.10±1.79	40.60±1.48	45.37±0.71
	100	17.40±1.02	21.60±1.18	27.10±1.37	35.30±1.42	42.10±0.90	44.00±0.70
Infected control (without treatment)	-	18.48±0.99	22.40±1.20	36.90±1.40	42.70±2.40	45.90±2.05	47.80±0.59

1) Mean of ten plants with ±S.E. in each case.

of other fungicides used also gave better yield compared to non treated infected control plant, but delan and sulfex reduced the yield considerably (Fig. 8.3).

DISCUSSION

In the present work fungistatic nature of some fungicides to sclerotia of **S. rolfsii** were recorded in the initial stage, their activity were also found to remain in the soil for a longer time. The non recovery of sclerotia from soil seems to be due to the toxic affect of fungicides, which penetrated into the sclerotium, resulting distintegration of the stored materials. The fungistatic nature of PCNB on **S. rolfsii** has also been reported by Chaudhuri and Maiti (1975). Reports are also available on the break down products of PCNB, which include penta chloro-aniline (PCA) and methyl thio pentachlorophenol, as a result of microbial activities in the soil (Chacko **et al**, 1966; Kaufman, 1970) and resulted in detoxification of PCNB. They observed for short term activity, and found the inhibition decreased with an increase in incubation period. In the present work, PCNB (i.e. 100 µg/L) also reduced sclerotial germination at the initial stage due to its inhibitory activity, but later due to detoxification, the sclerotia showed germination. Other fungicides tested also showed inhibitory effect on the sclerotial germination.

Fig.8.3: Effect of fungicides soil drench on the yield produced by soybean plants grown in the **Sclerotium rolfsii** infested soil

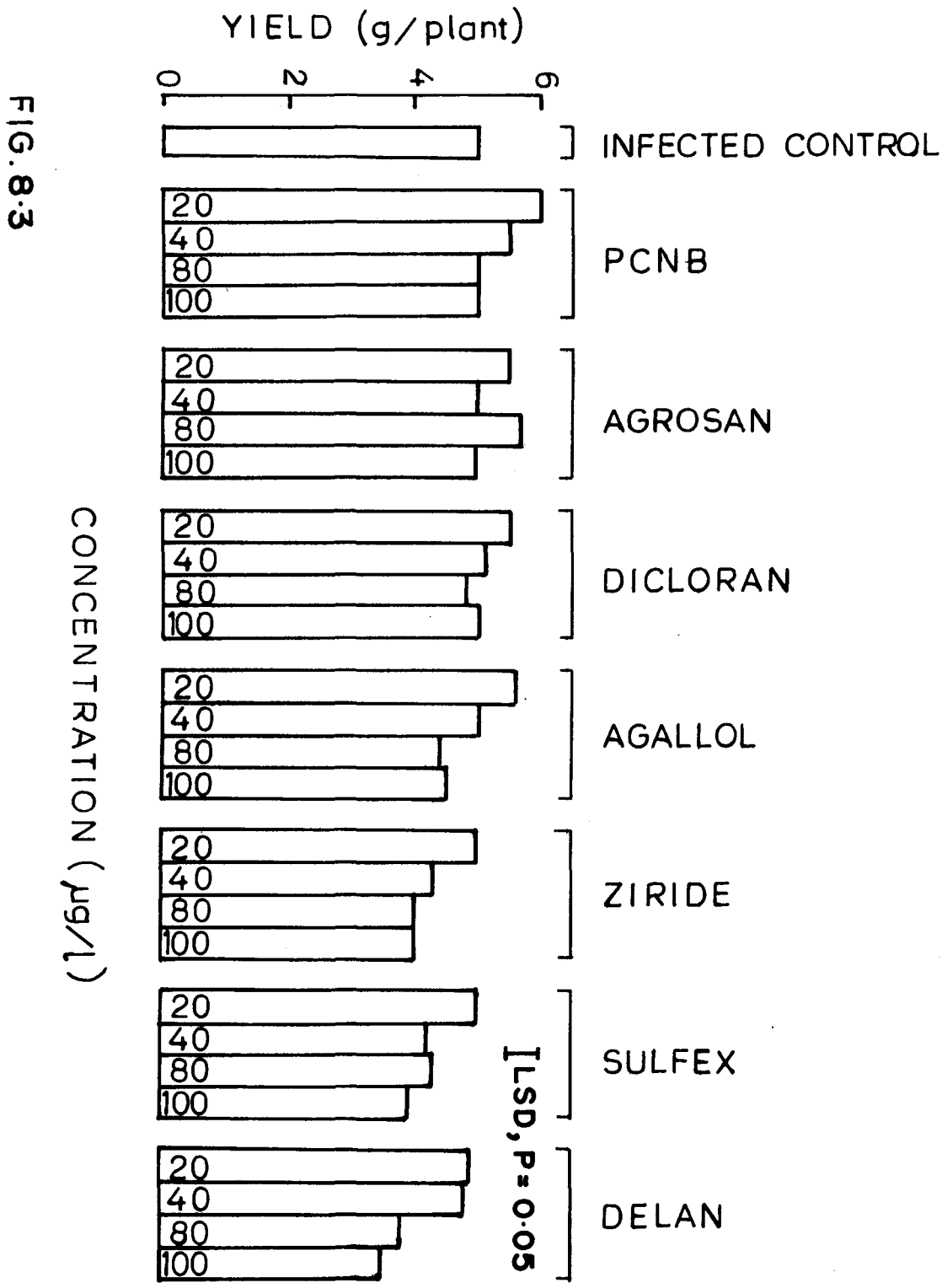

YIELD (g/plant)
0
2
4
6
INFECTED CONTROL
PCNB
AGROSAN
DICLORAN
AGALLOL
ZIRIDE
SULFEX
DELAN
20
40
80
100
CONCENTRATION (µg/l)
LSD, P=0.05

FIG. 8.3

The fungicides in the present experiment were found to be toxic against the pathogen but non-toxic to the host plant (except sulfex). This according to Dimond (1963) should be the quality of a therapeutant, and in practice he found this very difficult to achieve because, "the basic biochemistry of fungi and higher plants has much in common."

Soil drench reduced the microbial population in the rhizosphere of soybean in the present study which may be due to the toxicants or break down products of them, which might have had direct toxic effect on the rhizosphere microflora. Some of the fungicides also translocated systematically in the plant, resulting in a change in the host physiology and root exudates pattern, affecting the rhizosphere microflora.

The importance of the soil types exerting a distinct effect on the distribution of certain fungi, not only in the soil but also on the rhizosphere, was stressed by Peterson (1958). It could be concluded from the present work that the nature of fungal flora in the rhizosphere and the resultant stimulation of a particular genera of fungi i.e. **Aspergilli** and **Trichoderma** spp., after soil drench, seemed to be influenced partially by the soil types and therefore, the conclusion conform with those drawn by Peterson (1958). Thus it can be concluded that the fungicides had a profound effect on the distribution of rhizosphere microflora as well as the percentage occurrence of certain fungi.

Production of better yield of soybean in some cases (i.e. PCNB and agrosan) compared to infected control suggests that the fungicides affected the host metabolism favourably. On the other hand, delan, sulfex and higher concentration of agallol and ziride produced poor yield. It has frequently been suggested that a chemotherapeutant may operate indirectly by altering the host metabolism in such a way that they produce more resistance to disease. Widely differing mechanism by which this increased resistance can be brought about have been suggested by Dimond (1963, 1965) and Grossman (1968). These include alteration to (a) the host surface, (b) nature of the pectic substance (Edgington **et al**, 1961), (c) the wood morphology (Beckman, 1958), (d) carbohydrate levels (Horsfall and Dimond, 1957) and (e) phenolic constituents (Kaars Sijpesteijn and Pluijers, 1962; Holowczak **et al**, 1962).

Reduction in disease severity (50%) by almost all the fungicides at higher concentration, and delaying rot symptom expression in case of PCNB and agrosan, in the present work, may be due to the fact that the fungicides affect the pathogen directly and might have changed the host metabolism through systemic translocation. Similar results were also reported by Chaudhuri and Maiti (1975) with PCNB and suggested that PCNB has inhibitory effect on **S. rolfsii**, which delayed the onset of the disease, but never eradicated the pathogen (i.e. **S. rolfsii**) from the soil. Hence, a split in application

of this fungicide during the growing period of the crop was suggested by them.

CHAPTER VII

STUDIES ON THE ANTAGONISM OF SOME FUNGI ISOLATED FROM THE SOIL AND RHIZOSPHERE OF SOYBEAN PLANTS AND THE CONSEQUENT PROSPECT FOR THE CONTROL OF FOOT ROT DISEASE CAUSED BY SCLEROTIUM ROLFSII

INTRODUCTION

Several attempts have been made to control soil-borne diseases by introducing or by stimulating antogonistic micro-organisms in soil. Weindling (1932) demonstrated that **Trichoderma lignorum** (Tode) Harz. parasitised as well as inhibited the development of **Sclerotium rolfsii** mycelium and preliminary experiments indicated a degree of biological control.

The inhibition in growth and sclerotium production in case of **S. rolfsii** by microorganisms have been reported by many workers i.e. bacteria (Mehrotra and Caludius, 1972; Agrawal **et al**, 1977; Brathwaite and Cunningham, 1982), actino-mycetes (Mehrotra and Caludius, 1972; Singh and Reddy, 1979), mycorrhizal fungus (Krishna and Bagyaraj, 1983) and **Trichoderma** spp. (Mehrotra and Caludius, 1972; Agrawal **et al**, 1977; Arora and Dwivedi, 1979; Elad **et al**, 1980; Bell **et al**, 1982 and Henis **et al**, 1984). Although some of these microorganisms suppressed disease under controlled experimental condition, few studies only have demonstrated the efficacy of these agents for the control of **S. rolfsii** under field condition.

The antagonistic microorganism such as **Aspergillus** spp. (Shigemitsu **et al**, 1978) and **Trichoderma** spp. (Henis **et al**, 1983) are known to penetrate the melanized rind of sclerotia and to destroy the inner sclerotial tissues. **T. harzianum** reported to excrete β (1,3)-glucanase and chitinase when grown on mycelium and cell wall of either **S. rolfsii**

or **Rhizoctonia solani**, causing degradation. This followed by penetration of the antagonistic fungus into the mycelium of the test pathogen has also been observed under scanning and transmission electron microscopes (Elad **et al**, 1983a,b). Elad **et al** (1984), further observed that **T. harzianum** degraded the walls of sclerotial cells and thus the attacked cells lost their cytoplasmic contents, enabling them to sporulate on the sclerotial surface and inside the digested cells.

The application of antagonists by seed treatment or by fluid seed drilling procedure (Gray, 1981), are attractive methods for the introduction and establishment of bio-controlling agent in the infection site of the host seems interesting as both the treatments require smaller amount of inoculum than either broadcast or in furrow treatments (Harman **et al**, 1981; Klassen, 1981 and Elad **et al**, 1982).

The influence of plant growth by soil microbes has been emphasized by different researchers (Wright, 1954; Martin **et al**, 1956; Subba-Rao **et al**, 1961) and which possibly affect through the production or exudation of toxic or growth promoting substances or by the competition between plants and micro-organisms for the nutrients. The pathogenic root infecting fungi can survive saprophytically in soil or on organic matter by competing with obligate saprophytes or on dead host tissues invaded during the parasitic phase (Garret, 1975), determine the pattern of colonization by fungi and their ability to compete for substrates (Skidmore and Dickinson, 1976). Several

reports are available on the production of volatile metabolites by the cultures of **Trichoderma viride** (Dennis and Webster, 1971b), **T. harzianum** (Hutchinson and Cowan, 1972), **Fomes scutellatus, Fusarium oxysporum, Rhizopus stolonifer, Penicillium expansum** and **Geotrichum candidum** (Robinson and Park, 1966; Robinson and Garret, 1969). The substances were detected as acetaldehyde, Co_2, ethanol, n-propanol, propionaldehyde, isobutanol, ethyl acetate, isobutyl acetate, acetone etc., whereas non volatile metabolites usually diffuse into the substrate resulting inhibition in the growth of others. Mitchell (1973) reported that some of **Trichoderma** isolate produce non volatile antibiotic, active against a wide range of fungi. He further observed that the culture filtrates of **Trichoderma** reduce the spore germination of **Gliocladium roseum.** Saprophytic soil fungi have high competitive ability and undoubtedly play a major role in the soil. Thus the relatively vigorous and competitive growth habit of saprophytic organisms is evidence of their physiologically advanced nature, a fact which may be exploited in soil-borne plant disease control.

Isaac (1954) observed that **Blastomyces luteus** was antagonistic in culture to both **Verticillium albo-atrum** and **V. dahliae.** The culture filtrate of **B. luteus** also have inhibiting effect upon the growth of these two above mentioned species. It was observed earlier that the organic, inorganic

soil amendments and foliar sprays stimulated **Trichoderma, Penicillium, Aspergillus** and **Fusarium** spp. in the rhizosphere of soybean, and **in vitro** studies, some of these fungi were also found to be antagonistic to **S. rolfsii**. Therefore, in the present work, an attempt was made to determine the extent of interaction between these fungi and **S. rolfsii**, both in culture and in phytopathological experiments, in order to investigate the possibility of their uses in the control of foot rot disease.

The activity of different antagonists against the growth of **S. rolfsii** was investigated through the following experimentations,

(i) volatile and non-volatile antibiotic production,
(ii)hyphal parasitism,
(iii)effect of culture filtrates, and
(iv) by incorporating the antagonists into the **S. rolfsii** infested soil.

MATERIALS AND METHODS

Dominant rhizosphere fungi viz. **Trichoderma viride, T. harzianum, T. koningii, Aspergillus niger, A. candidus, A. flavus, A. versicolor, Penicillium rubrum, P. oxalicum, Fusarium solani** and **F. oxysporum** were isolated in pure form and different experiments were performed following the methods described below:

Non-volatile antibiotic production

An agar diffusion method of Dennis and Webster (1971a) with slight modification was employed. Sterilised PDA medium (15ml) was poured into sterile plates (90mm). A deplasticised and sterilised (in boiled water and then in alcohol) cellophane disk (90mm) was placed asceptically over the medium and the plates were left over night for evaporation of moisture. Inoculum discs of the test organisms were cut and inoculated over the cellophane in the centre and was incubated for 120 hours at 25±1°c. Thereafter, the cellophane along with adhering mycelial growth of test organisms were removed and 4 mm inoculum discs of the test pathogen (i.e. **S. rolfsii**) obtained from vigorously growing culture plates was placed immediately on the medium at the position previously occupied by an antagonist (i.e, test organism). The plates were again incubated and colony diameter was measured at an interval of 24, 48, 72 and 96 hours. The control set was inoculated with only virgin agar discs over cellophane instead of test organism. Percentage inhibition was calculated according to Isaac and Heale (1961).

Volatile antibiotic production

The method of Dennis and Webster (1971b) was adopted. Test organisms were grown on PDA in plates for a period of 10 days at 25±1°c. Thereafter, the lid of each petriplate was replaced by the same size of bottom plate containing

15ml (approx) PDA medium, centrally inoculated with 4mm inoculum disc of test pathogen. The two dishes were tapped together with adhesive cellotape. The lid of the control plates which had not been inoculated with test organism were also replaced in the same manner. The test plate and control were set up in replicates and was incubated at 25±1°c. The colony diameter of test pathogen was measured after 24, 48, 72 and 96 hours. Percentage of inhibition was calculated accordingly.

Colony interaction and hyphal interference between the test organism and test pathogen

A modified method of Ikediugwu and Webster (1970a,b) as described by Skidmore and Dickinson (1976) was used to study the hyphal interference.

A sterilised (in boiled water and then in alcohol) and deplasticised cellophane sheet (90mm) was placed over the plate containing PDA medium, and left overnight to evaporate excessive moisture. Inoculum disc (4mm) was cut from vigorously growing culture of test organism and the test pathogen were placed 2-3 cm apart on cellophane in dual culture and was incubated for 4-5 days at 25±1°c in dark. When the mycelium of both the fungal species came in close proximity a detailed microscopic examination were made for hyphal interference and mycoparasitism. For further detailed studies, a square of cellophane cut from the area of intermingling growth was

mounted on clean microscopic slide in lectophenol and cotton blue and was observed carefully under micro-scope. Photomicrographs of hyphal interactions were taken when required.

i) **Effect of antagonist's culture filtrate on the growth of the test pathogen** (i.e. S. rolfsii)

Disc (4mm) of each test organisms (i.e. antagonists) were inoculated in Czapeck dox's solution (100 ml per 250ml flask) separately, and was allowed to grow for ten days on a slow mechanical shaker at 25°c±1. The mycelial mats were then removed by passing the entire filtrate through whatman No.1 filter paper.

Two sets (sterilised and non-sterilised culture filtrates) each having five replicates were prepared for each fungus. 10 ml sterilised PDA medium was poured into each dish, and before the agar solidifies, 3 ml of the filtrate of the respective antagonistic fungus was added. Control was prepared similarly but with Czapeck dox's solution and PDA medium only and was kept separately. The plates were then inoculated with 4 mm disc of **S. rolfsii** and their radial growth was recorded after the incubation period (i.e. 24, 48, 72 and 96 hours respectively).

ii) **Effect of antagonists culture filtrate on the sclerotial germination**

Culture filtrate (5 ml in each case) was taken into test tubes containing 15 viable sclerotia of **S. rolfsii**, inoculated from freshly prepared cultures and was incubated

for 7 days at natural temperature. The percentage germinability of sclerotia was recorded following the method of Agnihotri et al (1975) as described in page 41.

iii) **Effect of antagonists culture filtrate on Soybean seed germination**

Surface sterilised soybean seeds (sterilised in 0.1% $HgCl_2$ solution) were soaked in the culture filtrates of antagonists, for 24 hours separately. The treated seeds of each set were then placed in moist chamber (sterilised petriplates containing moist filter paper) and 5 such replicates were kept for each treatment at 25±1°c (inside a BOD incubator). Control set was prepared with sterilised liquid medium and sterilised distilled water similarly. Percentage germination and radicle length of soybean seeds were recorded after 7 days of incubation.

Control of foot rot disease of soybean by antagonists

Pathogen infested natural soil was collected from ICAR farm, Barapani, Shillong, and to this freshly cultured test pathogen (i.e. **S. rolfsii** grown in sand wheat medium) was mixed (1% w/w) to ensure the increase of inoculum in the soil. Rhizosphere fungi (i.e. antagonists grown in sand wheat medium) were then mixed thoroughly (1% w/w of soil) in the experimental soil and was kept for 15 days in natural condition. Appropriate control i.e. without antagonists was kept for comparison. A minimum of three replicates were made for each treatment.

Soybean seedlings were raised in sterilised soil. Three weeks old seedlings were directly transferred to the antagonist amended infested soil (10 seedlings/pot) without injuring the root system. Control set was also prepared in similar manner. Percentage of infected plant together with soybean seed yield was recorded.

Since in the preliminary screening of the antagonists, **Trichoderma harzianum** and **T. koningii** gave better results compared to other microorganisms tested, a further detailed investigation with these two organisms were conducted. Different concentrations (i.e. 0.10, 0.25, 0.5% w/w) of the antagonists were mixed separately in the naturally infested soil (in 22cm diameter pots), in four replicates. After 15 days of natural incubation, soybean seedlings were raised from seeds (15 seeds/pot). Percentage of infected plants (calculation based on 40 plants), population of **S. rolfsii** in the antagonist amended soil (following slightly modified selective medium for **S. rolfsii** as proposed by Backman and Rodriguez-Kabana, 1976) together with soybean yield was recorded.

RESULTS

Volatile and non-volatile antibiotic production

Most of the fungi tested against the growth of **S. rolfsii** showed a wide range of variation in inhibition by producing volatile and non-volatile antibiotic like substances. Among the **Trichoderma** spp., **T. viride** and **T. koningii** were

found to be the most efficient producers of volatile antibiotics, whereas all the three species of **Trichoderma** tested (i.e. **T. viride, T. koningii** and **T. harzianum**) and **Aspergillus flavus** showed the highest non volatile antibiotic activity against the test pathogen (i.e. 80-96% inhibition in the growth of **S. rolfsii**). **Penicillium** spp. **Fusarium** spp. and other species of **Aspergillus** also showed inhibition but to a lesser extent (50-80%) (Fig. 9.1). The inhibitory effect caused by the volatile antibiotic or antibiotic like substances decreased with time (24-96 hours) excepting **T. viride** and **T. koningii** (Fig. 9.1).

Colony interaction and hyphal interference

Results on the colony interaction and hyphal interference between the selected soybean rhizosphere fungi and **S. rolfsii** were presented in the Table 7.1. **Trichoderma koningii** and **T. harzianum** inhibited the growth and grew over the colony of **S. rolfsii** ('B ii' type of antagonism according to the classification of Skidmore and Dickinson, 1976), which subsequently covered the colony of the later completely (Plate 16). **T. koningii** shows coiling only to the **S. rolfsii** mycellium (Plate 17), whereas penetration, lysis, bursting of hyphae were detected (Plate 18) in case of **T. harzianum**. It was also initially observed that the **T. harzianum** conidia were attached to the hyphal wall of **S. rolfsii**, resulting absorption of nutrients, possibly

Fig.9.1: Effect of volatile and non-volatile antibiotic producing microorganisms on the growth of **Sclerotium rolfsii in vitro.**

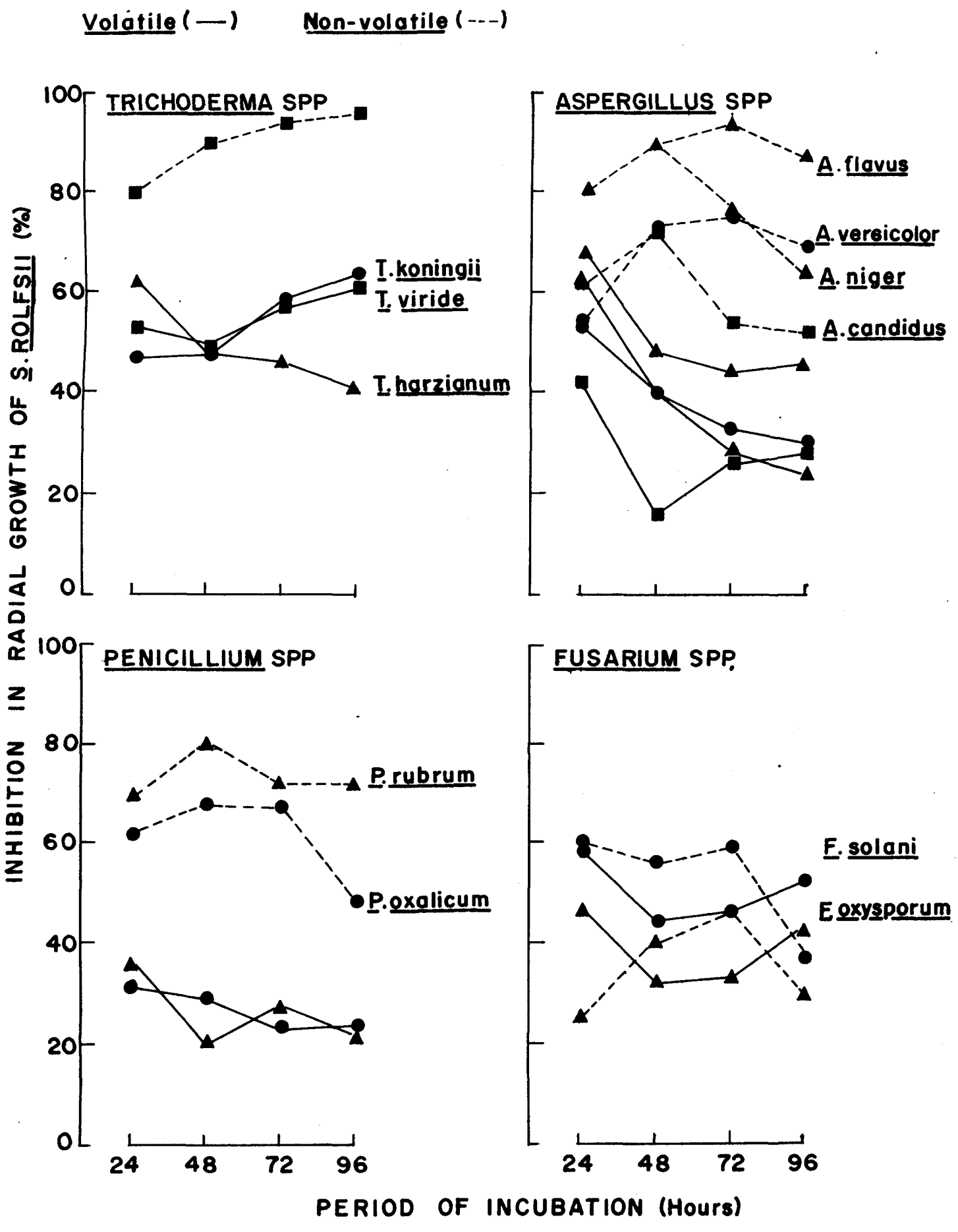

Volatile (—)
Non-volatile (---)
TRICHODERMA SPP
T. koningii
T. viride
T. harzianum
ASPERGILLUS SPP
A. flavus
A. versicolor
A. niger
A. candidus
PENICILLIUM SPP
P. rubrum
P. oxalicum
FUSARIUM SPP.
F. solani
F. oxysporum
INHIBITION IN RADIAL GROWTH OF S. ROLFSII (%)
0
20
40
60
80
100
24
48
72
96
PERIOD OF INCUBATION (Hours)

Fig. 9·1

TABLE 7.1 : Colony interaction and hyphal interference caused by the rhizosphere fungi of soybean against **S. rolfsii.**

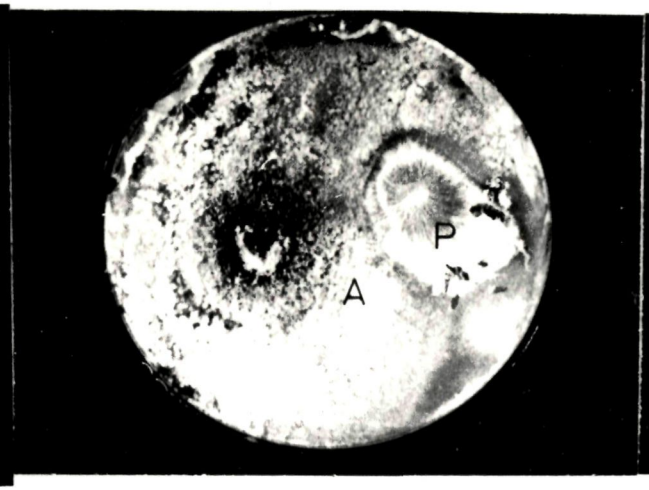
Organisms	Colony interaction	Hyphal Interference
Trichoderma koningii	Bii	Coiling
T.viride	C	Coiling, penetration, growth, lysis, bursting, conidium formation, absorption of nutrients by conidia.
T. harzianum	Bii	Penetration, lysis, absorption of nutrients by conidia, bursting.
Aspergillus niger	Bi	Coiling, penetration, growth, lysis.
A. flavus	Bii	Not detected
A. candidus	ND	Not detected
A. versicolor	A	Absent
Fusarium solani	C	Absent
F. oxysporum	A	Not detected
Penicillium rubrum	Bi	Absent
P. oxalicum	A	Absent

A. Mutual intermingling growth; Bi. Over growth by antagonist; Bii. Intermingling by which the fungus under observation has ceased growth and is overgrown by another colony; C. Slight inhibition; ND. Not detected.

Plate 16: Showing the colony interaction between the antagonists (A): (1) **Trichoderma viride,** (2) **T. harzianum,** (3) **T. koningii,** (4) **Aspergillus niger,** (5) **A. versicolor,** (6) **A. flavus** and the test pathogen (P) **Sclerotium rolfsii.**

PLATE-16

1



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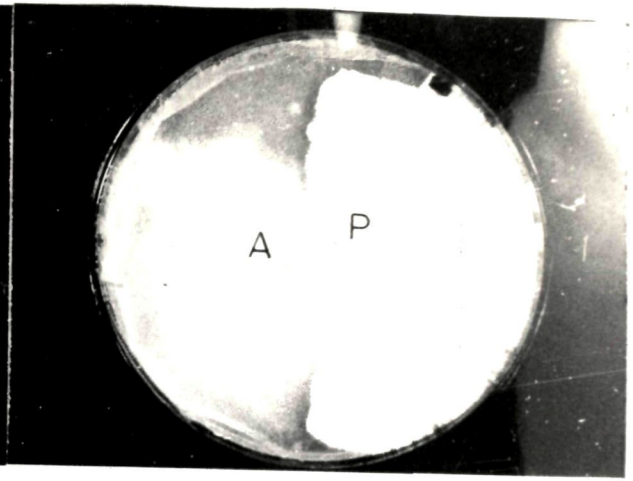
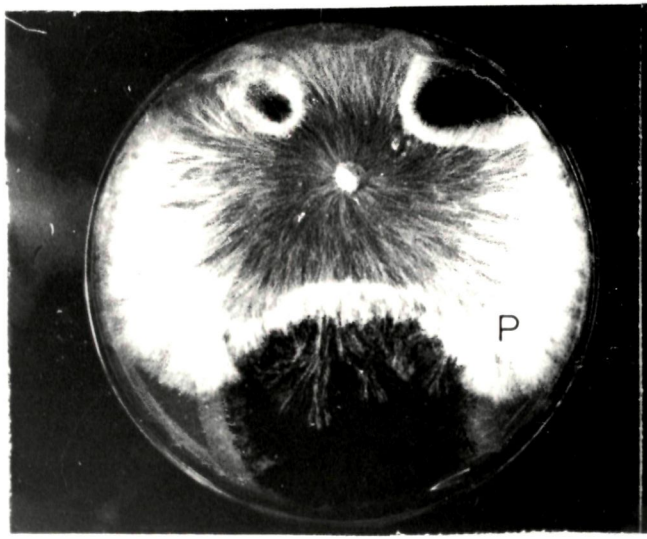
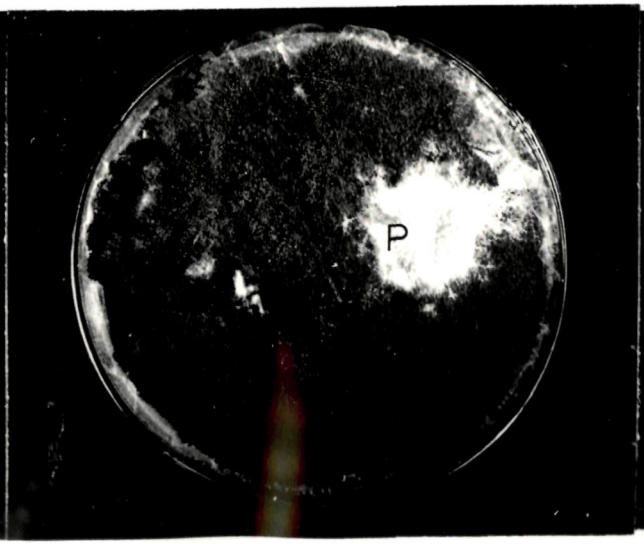


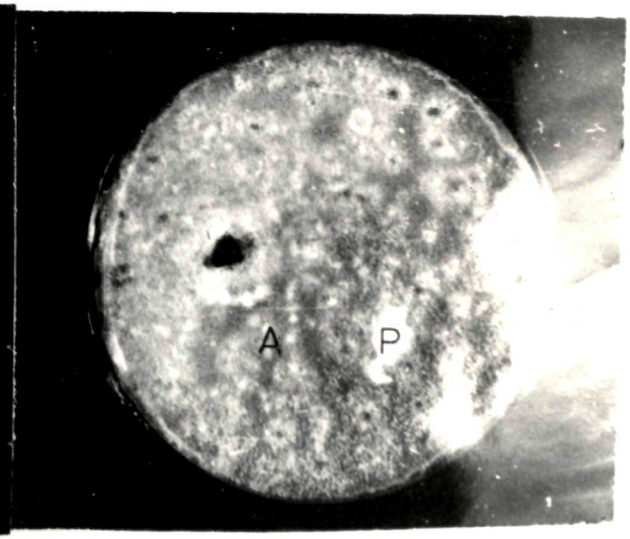
3



4



5



6

Plate 17: Showing the coiling of the hypha of (a) **Sclerotium rolfsii** by (b) **Trichoderma koningii** (X 400).

PLATE-17

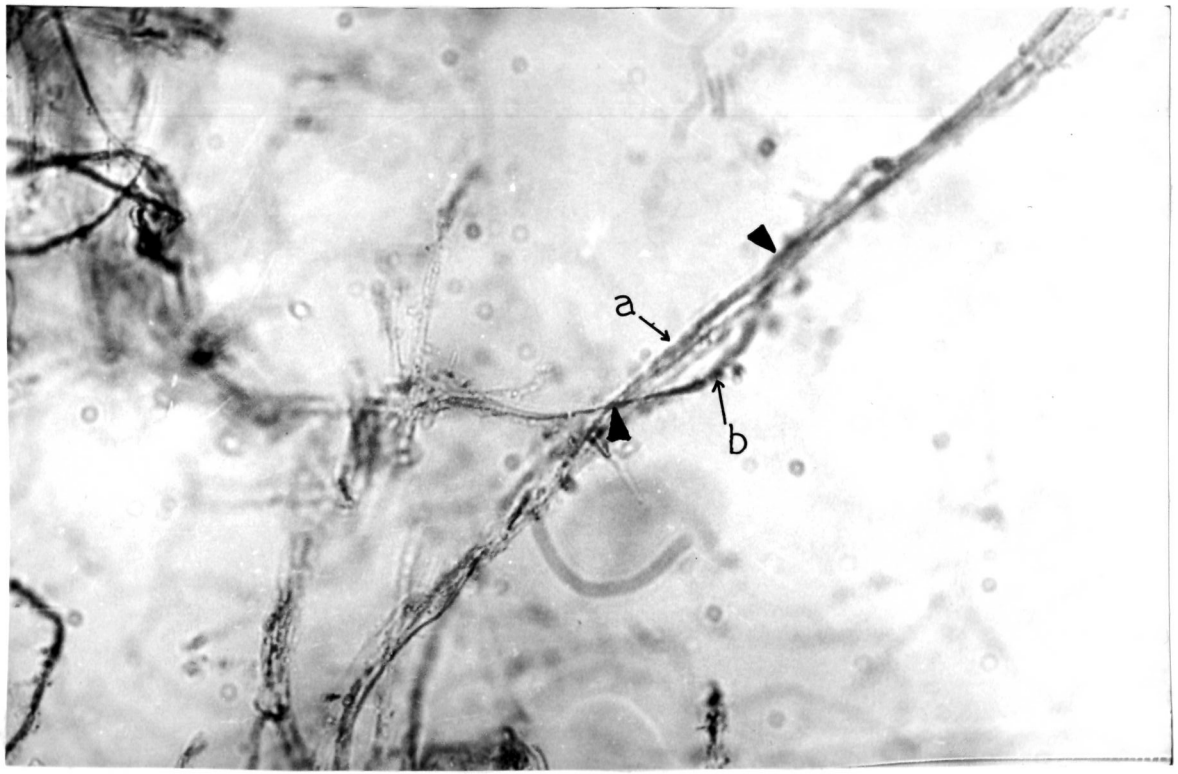


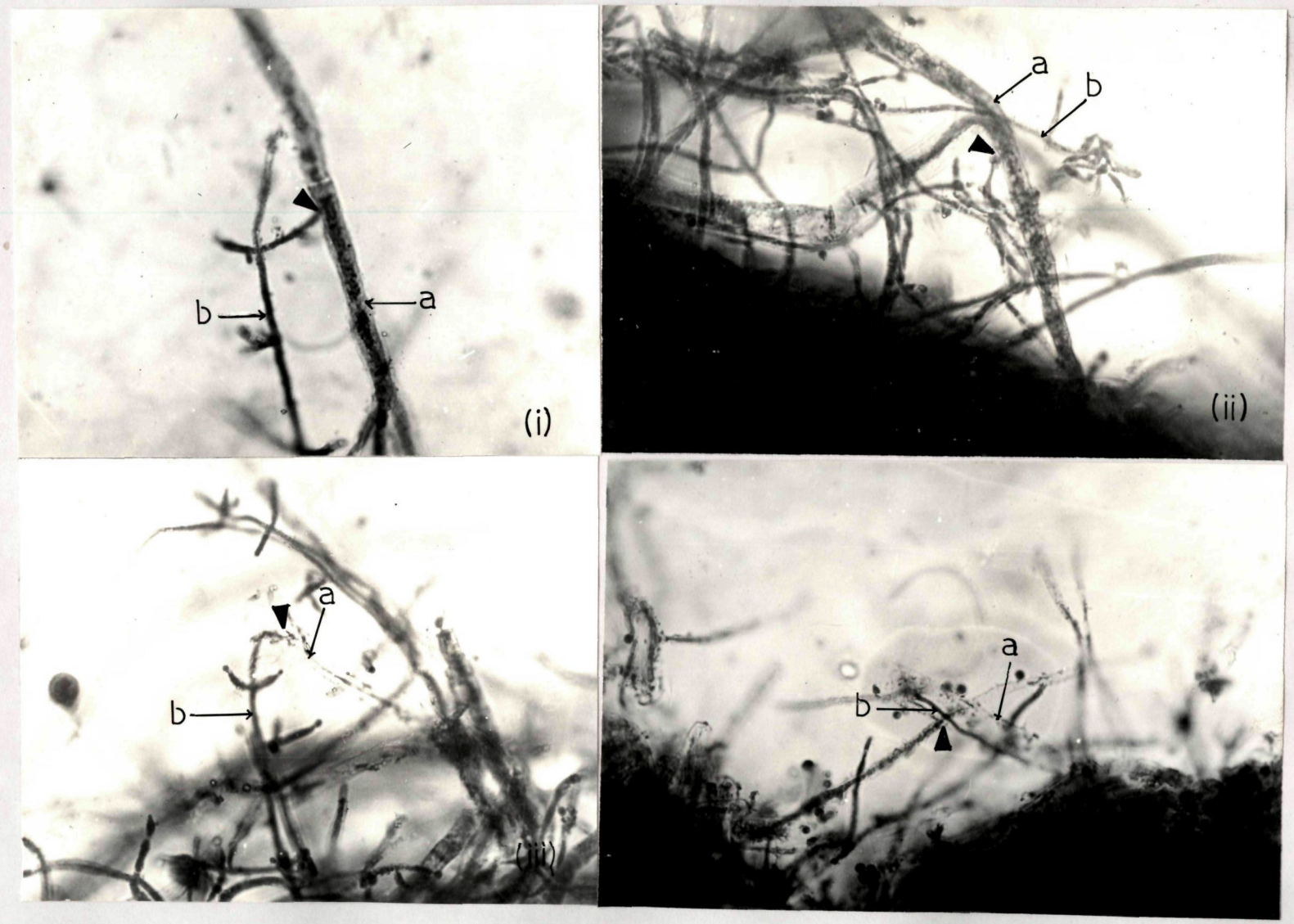
Plate 18: Showing the hyphal parasitic interaction between (a) **Sclerotium rolfsii** and (b) **Trichoderma harzianum.**

Fig. (i) Showing the penetration of **T. harzianum** into the hypha of **S. rolfsii** (X 400).

Fig.(ii) Absorption of nutrient by the conidia of **T. harzianum** (X 400).

Fig.(iii) & (iv) Lysis and bursting of the hypha of **S. rolfsii** by the hyphae of **T. harzianum** (X 400).

PLATE -18



together with the release of enzymes (Elad **et al**, 1983a,b) resulting disintegration of hyphal wall, followed by penetration etc. (Plate 18).

T. viride was found to have produced clear inhibition zone (Type 'C') (Plate 16). Although, this zone was distinctly visible but in some cases a slight overgrowth by **T. viride** in the periphery of the colony was also noticed. A close observation on the hyphal interference revealed that **T. viride** also parasitized the hyphae of **S. rolfsii** through coiling, penetration, lysis, formation of conidium inside (in some cases) resulting bursting of the hyphae (Plate 19). This parasitic behaviour of the antagonistic fungi have been categorised as the necrotrophic type (Barnett and Binder, 1973).

An interesting mycoparasitic activity of **S. rolfsii** on **Aspergillus niger** was also observed. In this case **S. rolfsii** inhibited the growth of **A. niger** by the formation of a thick mycelial mats around the colony, and grew over it ('B i' type; Plate 16). Coiling, penetration and growth inside the conidiophore and in some cases lysis of the **A. niger** was also observed. It was observed that **S. rolfsii** attack only conidiophores of **A. niger** (Plate 20).

A. flavus restricted the growth of **S. rolfsii**, and since it grow comparatively faster, it completely overgrew

Plate 19: Showing hyphal parasitic interaction between (a) **Sclerotium rolfsii** and (b) **Trichoderma viride.**

Fig.(i) Showing the overlapping and growth of **T. viride** around the **S. rolfsii** hypha (X 400).

Fig.(ii) Penetration of **T. viride** into the hyphae of **S. rolfsii** (X 400).

Fig.(iii) Absorption of nutrient by the conidia of **T. viride** from the hyphae of **S. rolfsii** (X 400).

Fig.(iv) Lysis of the hyphae of **S. rolfsii** by **T. viride** (X 400).

Fig.(v) Lysis and bursting of the hyphae of **S. rolfsii** by **T. viride** (X400).

Fig.(vi) Formation of conidia by **T. viride** inside the hypha of **S. rolfsii** (X 400).

PLATE-19

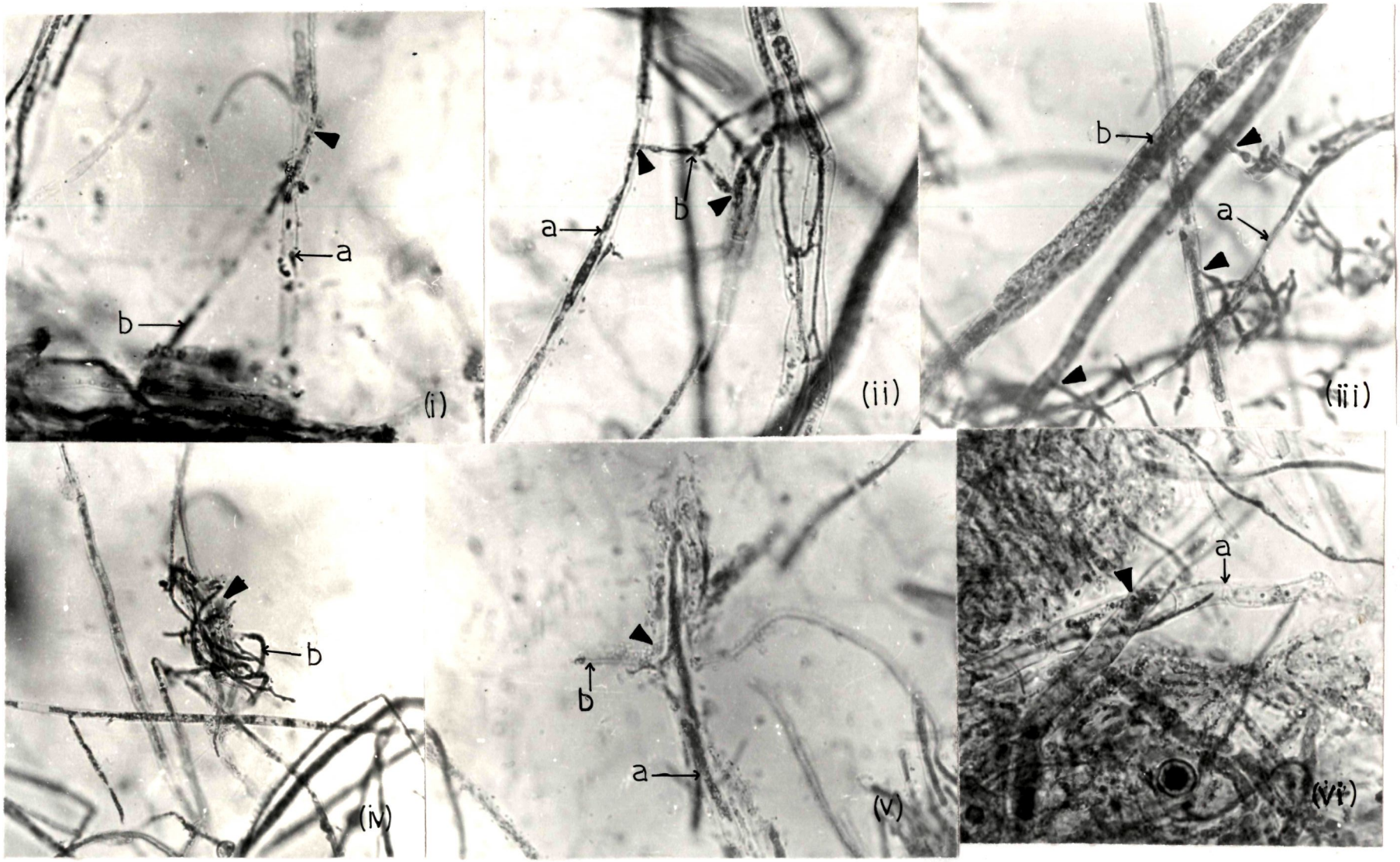
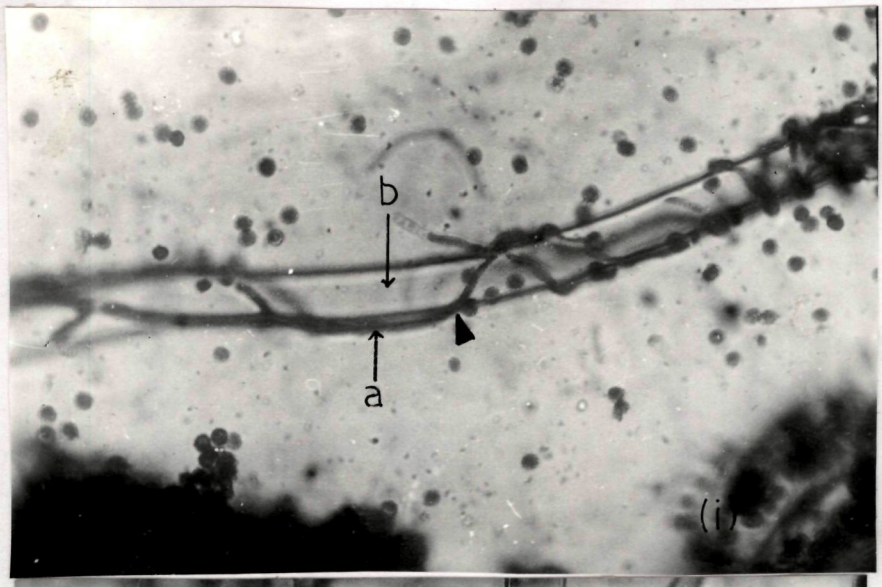


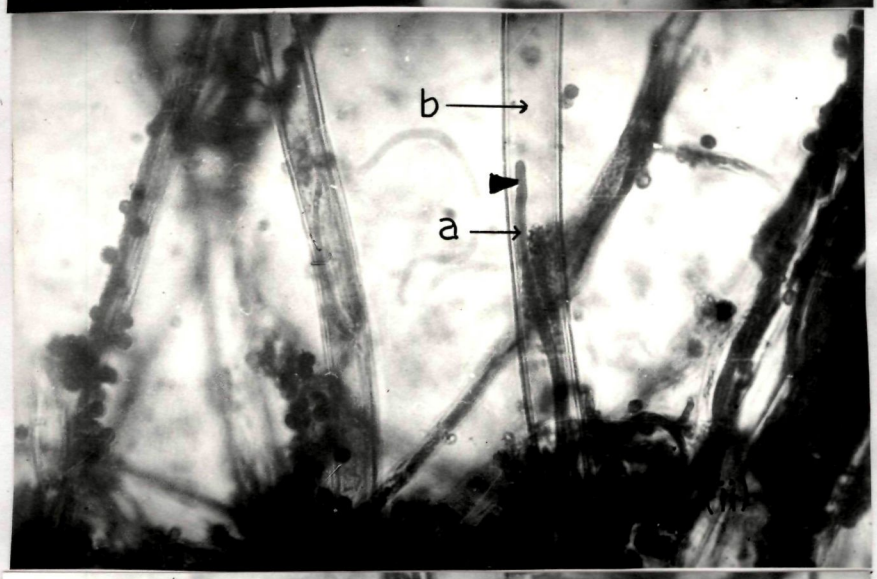
Plate 20: Showing the hyphal parasitic behaviour of (a) **Sclerotium rolfsii** on (b) **Aspergillus niger.**

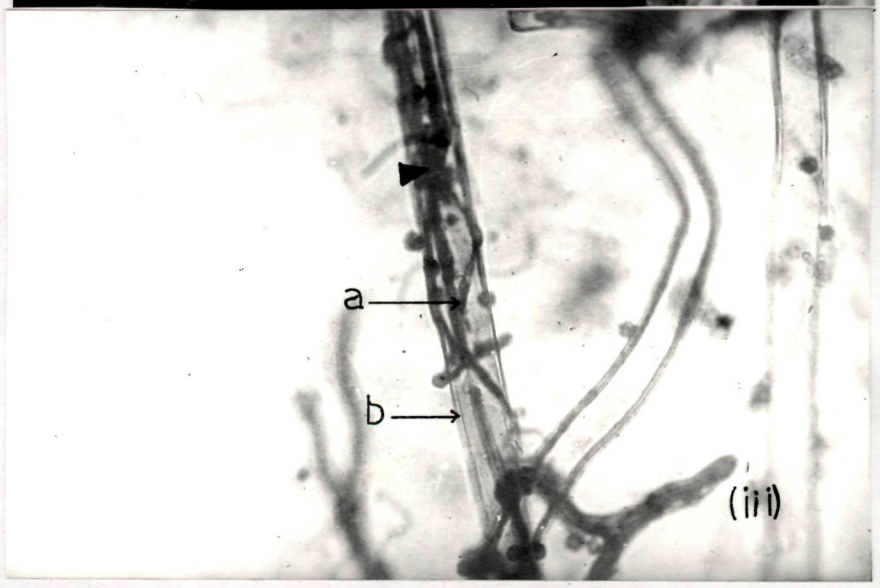
Fig.(i) Coiling of the conidiophore of **A.niger** by the hypha of **S.rolfsii** (X 400).

Fig.(ii) & (iii) Growth and branching of the hyphae of **S. rolfsii** within the conidiophore of **A. niger** (X 400).

PLATE-20







the colony of **S. rolfsii** ('B ii' type). Hyphal interference was not detected in this case. **A. versicolor** showed mutual intermingling of growth (Type 'A') whereas, **A. candidus** did not produce any interaction and hyphal parasitism (Table 7.1). Among the two species of **Fusarium : F. oxysporum** showed type 'A' and **F. solani** showed type 'C' kind of colony interaction. **F. solani** produced metabolites which changed the colour of the medium and a slight inhibition zone was also observed at the region of contact. Hyphal parasitism was not detected.

Although, Type 'A' and 'B i' colony interaction against **S. rolfsii** by **Penicillium oxalicum** and **P. rubrum** was observed respectively, no detection of hyphal interference was made (Table 7.1). Germination of sclerotia of **S. rolfsii** was also observed, when placed against the test organisms **in vitro.** The growth of the developed mycelium from the germinated sclerotia, was however inhibited due to the faster growth, as well as due to the metabolites released by the test organisms (i.e. antagonists).

Effect of antagonists culture filtrate on the growth of S. rolfsii in vitro

(i) The culture filtrate of the rhizosphere microflora (i.e. antagonists) showed inhibition to the growth of **S. rolfsii in vitro.** The rate of inhibition was more with the non sterile compared to the sterilised culture filtrate

in all the cases. Among the culture filtrates tested, **Fusarium** spp. was the most effective, in controlling the growth of **S. rolfsii** (Fig. 9.2D) followed by **Penicillium** (Fig. 9.2C) and **Trichoderma** spp. (Fig. 9.2B). **Aspergillus** spp culture filtrate also showed inhibition but to a lesser extent compared to others. *Sclerotial germination was also reduced by culture* filtrates (Table 7.2).

Effect of antagonist culture filtrates on the germination and radicle growth of soybean seeds

Slight effect on soybean seed germination was observed due to culture filtrate treatments. **Trichoderma koningii** culture filtrate infact was found to be stimulatory to the seed germination compared to others. **Aspergillus niger, A. versicolor, A. candidus** and **Penicillium rubrum** culture filtrate reduced the radicle growth, while filtrates of **Fusarium** spp. found to increase the same (Table 7.2).

Control of foot rot disease of soybean with the help of antagonists

Most of the antagonists tested in the present work reduced the disease severity (Table 7.3). The rot symptom appeared after three weeks of transplantation in the antagonists amended infested control soil. Among the eleven rhizosphere fungi selected for biological control studies, **Trichoderma harzianum** and **T. koningii** gave the best control of disease (26.7% and 36.6% infection respectively). The onset of disease

Fig.9.2 Effect of antagonist culture filtrates (sterilised and non sterilised) on the growth of **Sclerotium rolfsii in vitro.**

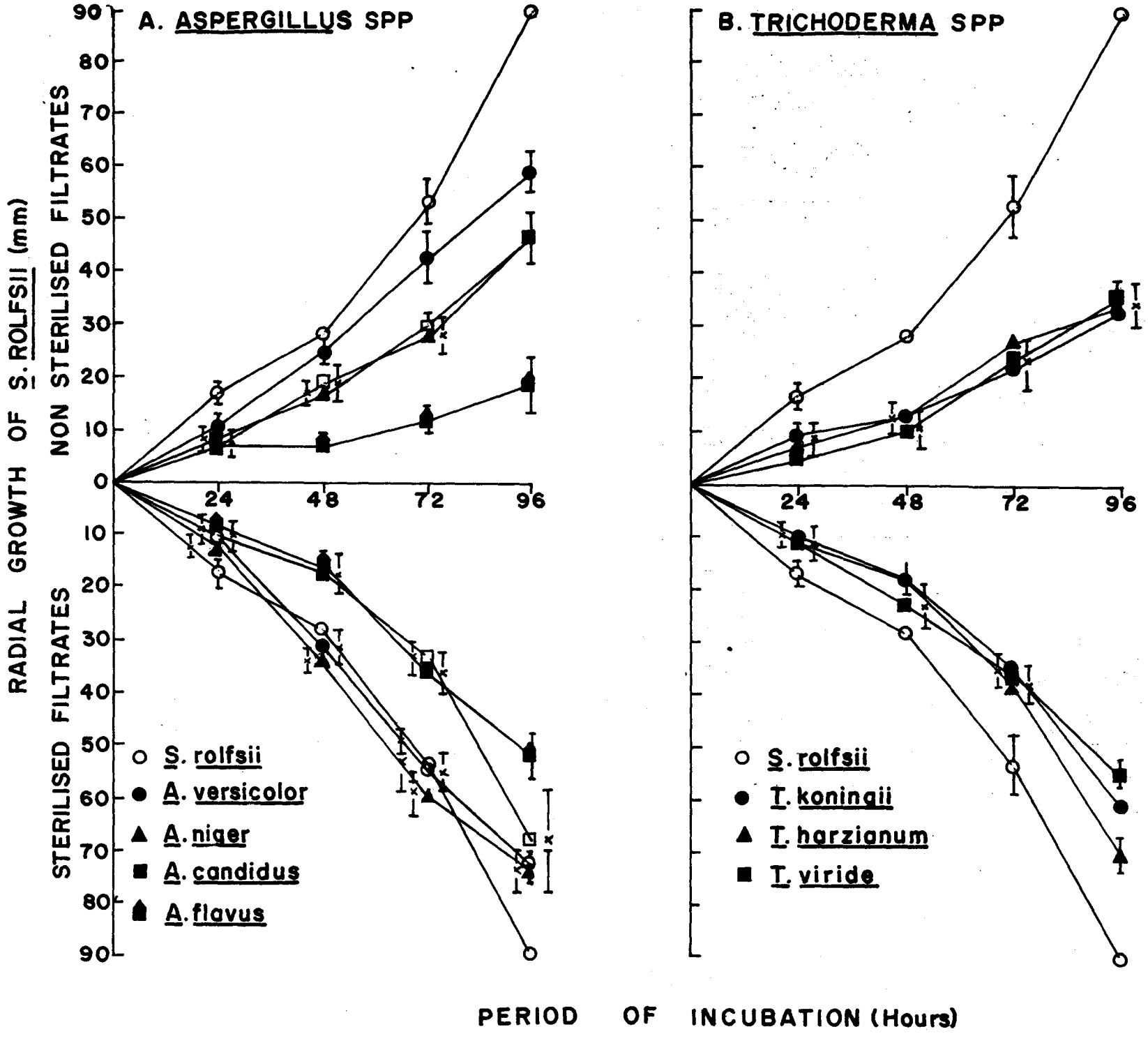

A. ASPERGILLUS SPP
B. TRICHODERMA SPP
RADIAL GROWTH OF S. ROLFSII (mm)
NON STERILISED FILTRATES
STERILISED FILTRATES
90
80
70
60
50
40
30
20
10
0
24
48
72
96
S. rolfsii
A. versicolor
A. niger
A. candidus
A. flavus
S. rolfsii
T. koningii
T. harzianum
T. viride
PERIOD OF INCUBATION (Hours)

Fig. 9·2

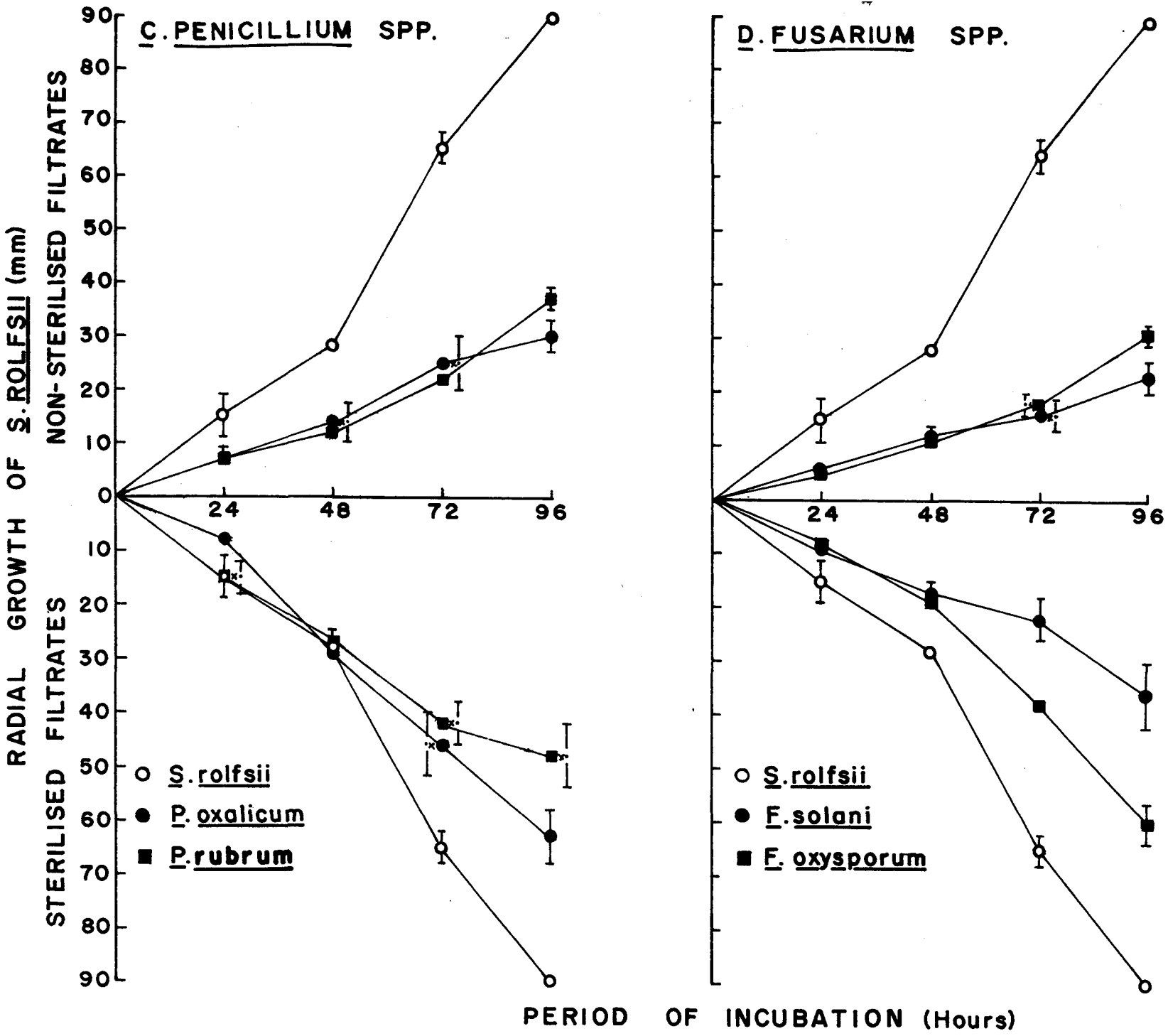

C. PENICILLIUM SPP.
D. FUSARIUM SPP.
RADIAL GROWTH OF S. ROLFSII (mm)
NON-STERILISED FILTRATES
STERILISED FILTRATES
90
80
70
60
50
40
30
20
10
0
24
48
72
96
S. rolfsii
P. oxalicum
P. rubrum
S. rolfsii
F. solani
F. oxysporum
PERIOD OF INCUBATION (Hours)

Fig. 9·2

TABLE 7.2 : Effect of antagonist culture filtrates on the sclerotial germination of **S. rolfsii**, soybean seed germination and radicle growth.

Antagonists	Sclerotium germination (%)[1]	Soybean seed germination (%)[2]	Radicle length (cm)[2]
Trichoderma koningii	60.00	86.70	7.25±0.80
T. viride	33.30	73.30	6.25±0.65
T. harzianum	33.30	70.00	6.80±1.07
Aspergillus niger	53.30	50.00	5.57±0.65
A. flavus	66.70	50.00	8.00±1.29
A. candidus	80.00	50.00	3.92±0.89
A. versicolor	80.00	60.00	6.00±1.06
Fusarium solani	53.30	73.30	8.62±1.04
F. oxysporum	40.00	50.00	8.57±1.23
Penicillium rubrum	46.70	60.00	4.86±1.18
P. oxalicum	40.00	60.00	7.14±1.48
Control (Czapeck dox)	100.00	80.00	6.88±1.45
Water	100.00	70.00	8.25±1.32

1) Calculations based on 15 sclerotia in each case.

2) Mean of 30 replicates with ±S.E.

TABLE 7.3 : Effect of soil amendments with antagonist cultures on the disease development caused by **S. rolfsii** in soybean plants.

Antagonists	Concentration (%)[1]	Percentage of Infection/week[2]				
		6	8	10	12	14
Trichoderma koningii	1	-	6.70	16.70	30.00	36.60
T. viride	1	10.00	26.70	36.70	50.00	50.00
T. harzianum	1	-	6.70	13.30	23.30	26.70
Aspergillus niger	1	6.70	16.70	23.30	33.30	46.70
A. flavus	1	13.30	33.30	46.70	53.30	66.70
A. candidus	1	6.70	20.00	23.30	40.00	50.00
A. versicolor	1	16.70	33.30	43.30	43.30	50.00
Fusarium solani	1	16.70	26.70	40.00	50.00	56.70
F. oxysporum	1	20.00	36.70	46.70	53.30	63.30
Penicillium rubrum	1	13.30	23.30	36.70	46.70	63.30
P. oxalicum	1	6.70	16.70	26.70	36.70	46.70
Infected control (without antagonists)	-	20.00	33.30	50.00	63.30	80.00

1) Percentage calculated in terms of grams (w/w).
2) Calculation based on 30 plants in each case.

in soybean plant was delayed in case of antagonist, **T. harzianum** and **T. koningii** amendments. **Fusarium** spp. amendment to soil, although showed an increase in infection rate of the initial stage, but latter a reduction of disease compared to control was observed.

Subsequent experimentation with promising antagonists i.e. **T. harzianum** and **T. koningii** (as determined from the earlier experiment) **in vivo** showed 45% and 35% reduction in disease severity respectively. The highest control of the disease was obtained with **T. harzianum** (i.e. at 0.5% w/w) amended to the **S. rolfsii** infested soil. **T. koningii** also showed reduction in disease severity in a similar manner (Fig. 9.3). Reduction of disease severity with the increase in concentration of the antagonists inoculum was also recorded.

No adverse affect on the growth of soybean plant was observed due to the antagonist amendments. Plants were much healthier, excepting in **Fusarium solani** and **F. oxysporum** amended soil. A comparative increase in plant growth (i.e. height and vigour) in case of **T. harzianum** amendment to soil was observed (Plate 21).

Population dynamics of Sclerotium rolfsii in antagonist amended soil

The population of **S. rolfsii** in the antagonist amended soil decreased considerably compared to the nonamended infested control. Reduction in population by 50% was recorded in

Fig.9.3: Effect of (A) **Trichoderma harzianum** and (B) **Trichoderma koningii** amendments to soil on the disease development in soybean, caused by **Sclerotium rolfsii.**

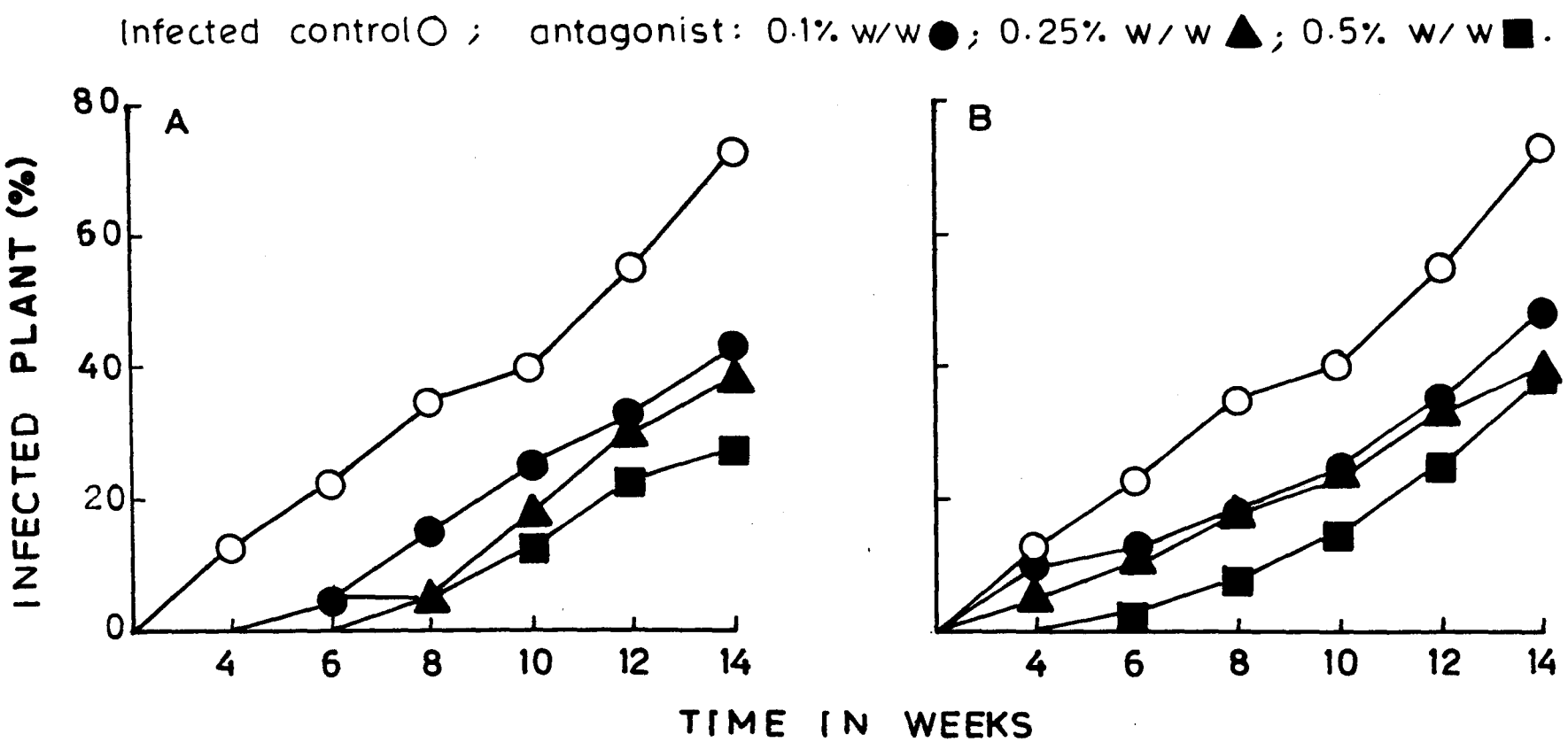

Infected control ○ ; antagonist: 0.1% w/w ● ; 0.25% w/w ▲ ; 0.5% w/w ■ .
A
B
INFECTED PLANT (%)
80
60
40
20
0
4
6
8
10
12
14
TIME IN WEEKS

FIG. 9·3

Plate 21: Showing the reduction in disease severity in soybean plants following **Trichoderma harzianum** amendment to soil (Infected control, 0.1, 0.25, 0.5% w/w of antagonist, left to right).

PLATE-21

most of the case (Table 7.4). Inoculum density of **S. rolfsii** increased at a slower rate, which was found to be directly related to the slow increase in disease severity. Reduced population of **S. rolfsii** in **T. harzianum** amended soil suggests their direct parasitic activity on the resting structures as well as on the mycelium, as evidenced from **in vitro** studies. This perhaps directly contribute towards the biological control of **S. rolfsii** in soil.

Effect on yield

Soybean seed yield increased due to the antagonistic fungi amendments to soil, in general, excepting **Fusarium solani** and **F. oxysporum**. Among all the species of **Trichoderma**, **T. harzianum** in particular, produced highest yield (7.0g/plant) (Fig. 9.4). In the subsequent experiment also a similar increase in yield was observed due to **Trichoderma harzianum** (0.1, 0.25, 0.5% w/w) amendment to infested soil (7.5, 7.7 and 7.0g/plant respectively) much higher than that of infected control (5.5g/plant). **T. koningii** in all the concentrations used (i.e. 0.1, 0.25, 0.5% w/w) were also found to increase the yield (Fig. 9.5). In general **Trichoderma harzianum** and **T. koningii** amendments gave the best control of the disease with increased soybean yield compared to infected control.

TABLE 7.4 : Population dynamics($x10^4 g^{-1}$ dry soil)[1] of **S. rolfsii** in antagonist amended soil.

Antagonists	Concentration (%)	Sampling period (days)			
		30	60	90	120
Trichoderma harzianum	0.10	1.70	1.67	2.04	2.75
	0.25	1.08	1.10	1.70	2.63
	0.50	1.08	1.00	1.38	2.46
Trichoderma koningii	0.10	1.78	1.96	2.50	3.20
	0.25	1.61	1.80	2.01	2.90
	0.50	1.58	1.75	2.10	2.50
Infected control (without antagonist)	-	2.80	4.04	4.54	5.30
LSD(P=0.05)	-	0.92	1.89	0.83	1.79

1) Mean of three replicates in each case.

Fig.9.4: Yield produced by soybean plants grown in the antagonistic fungi amended natural soil (infested with **S. rolfsii**) compared to untreated control.

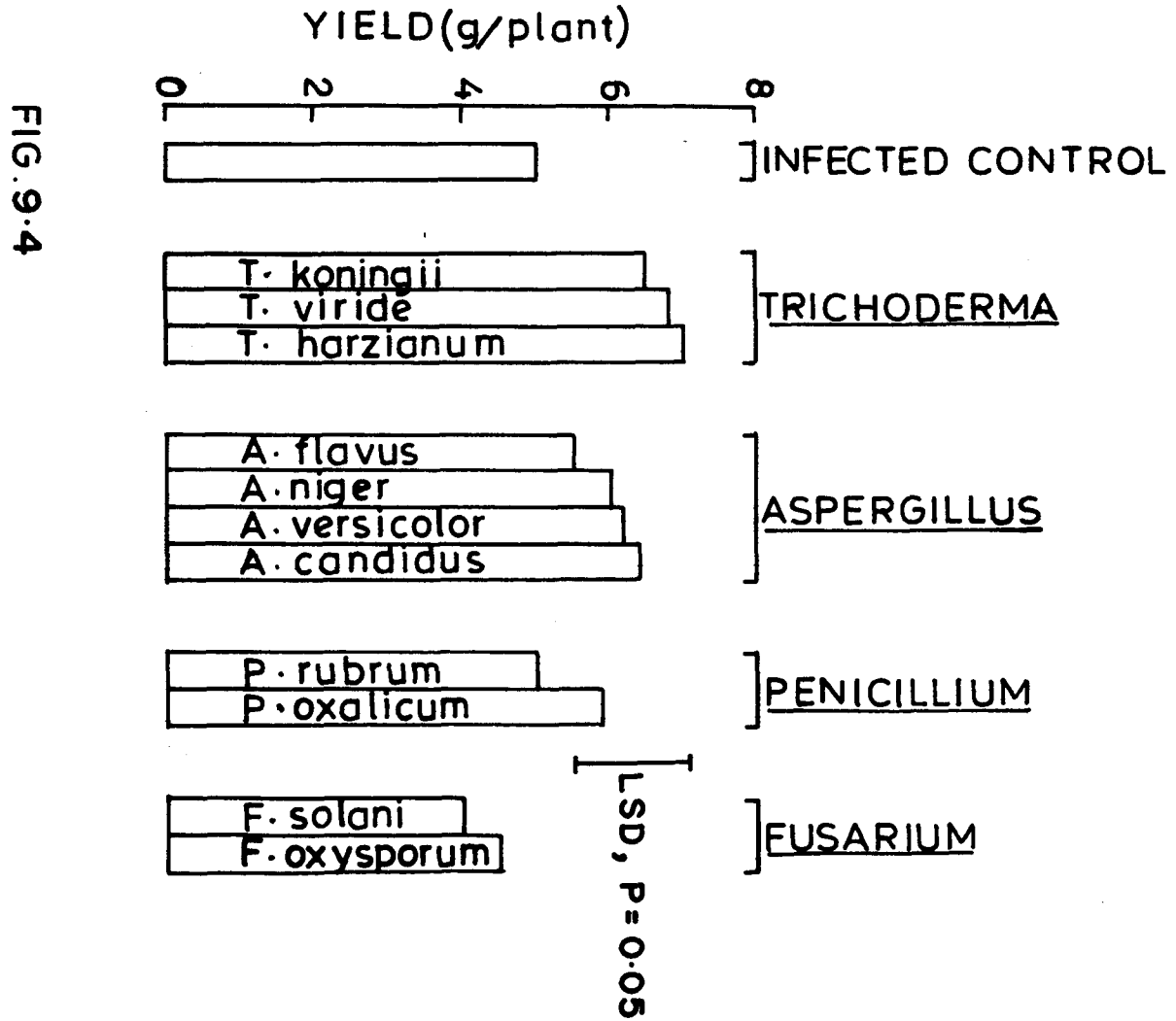

YIELD(g/plant)
0
2
4
6
8
INFECTED CONTROL
T. koningii
T. viride
T. harzianum
TRICHODERMA
A. flavus
A. niger
A. versicolor
A. candidus
ASPERGILLUS
P. rubrum
P. oxalicum
PENICILLIUM
F. solani
F. oxysporum
FUSARIUM
LSD, P=0.05

FIG. 9.4

Fig.9.5: Effect of antagonist (i.e. **Trichoderma harzianum** and **Trichoderma koningii)** amendments, on the yield of soybean plants grown in the **Sclerotium rolfsii** infested soil.

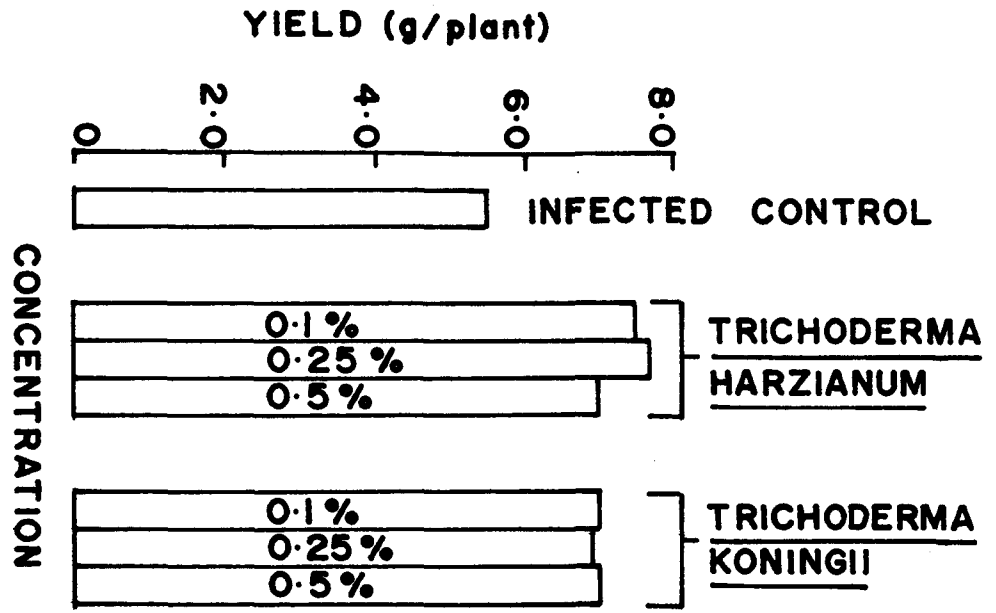

YIELD (g/plant)
0
2·0
4·0
6·0
8·0
INFECTED CONTROL
CONCENTRATION
0·1 %
0·25 %
0·5 %
TRICHODERMA HARZIANUM
0·1 %
0·25 %
0·5 %
TRICHODERMA KONINGII

Fig. 9·5

DISCUSSION

Much importance on the microbial antagonism has been paid more recently, mainly to reduce the dependency on the fungicides in agriculture, which causes ecological hazards in the process, has been the subject of growing concern to both environmentalists and public health authorities. Control of soil-borne plant diseases **in vitro** were observed by several workers (Mehrotra and Caludius, 1972; Agrawal **et al**, 1977; Singh and Reddy, 1979) but under field conditions the success has not been very encouraging. As in the former case, a controlled climatic condition such as temperature, water regimes, humidity, could be maintained properly which is quite often not possible under the field conditions.

Fungi present in the soil and rhizosphere mostly survived saprophytically and release substances which are either volatile and/or non volatile in nature. These substances may be either growth promoting substances, vitamins which induces plant growth or antibiotics which may inhibit the normal growth of the pathogen (Hopkins and Cole, 1903; Roberts and Roberts, 1939; Cook and Lockhead, 1959; Brain, 1960 and Baker and Cook, 1974). Some of them have parasitic activity on other microorganisms too. In the present study, among the rhizosphere fungi, **Trichoderma koningii** and **T. viride** produced volatile antibiotics/antibiotic like substances

and resulted in inhibition in the growth of **S. rolfsii**. Some rhizosphere fungi also produced non-volatile antibiotic like substances, which might have been diffused to the medium resulting in inhibition in growth of the test pathogen. **Trichoderma** spp. and **Aspergillus flavus** was the most effective among the fungi tested, in controlling the growth of **S. rolfsii in vitro**. The rate of inhibition decreased following the increase in incubation period, suggesting that the metabolites released were possibly volatile antibiotic like substances and might have gradually lost the inhibitory properties and possibly due to this reason most of the rhizosphere fungi inhibited the growth of **S. rolfsii in vitro** at the initial stage.

Experiment with the culture filtrates of the antagonistic fungi have showed that the metabolites produced by them in the liquid medium were inhibitory to the growth of the test pathogen (i.e. **S. rolfsii**). Sterilised filtrates also reduced the growth but to a lesser extent, suggesting the metabolites released by them were volatile as well as non volatile antibiotic/antibiotic like substances. It was reported earlier by several workers that **Trichoderma** spp. produces volatile and non volatile substances in culture (Dennis and Webster, 1971 a,b; Hutchinson and Cowan, 1972). The antibiotic action has been reported to be important in controlling **S. rolfsii** by **Pseudomonas aeruginosa** associated

with the production of pyocianine (Brathwaite and Cunningham, 1982). In the present investigation no detection was made on the nature of antibiotic/antibiotic like substances that were produced by the antagonists **in vitro.**

Seed germination and radicle growth was not affected by the culture filtrate of **Trichoderma** spp. Increased radicle growth by **Fusarium** spp. and **Trichoderma** spp. proved their stimulatory effect. This further suggests that these rhizosphere fungi might play an important role in plant growth and therefore needs further attention.

Most of the rhizosphere fungi isolated in the present investigation, survive saprophytically in nature. In culture medium they grew very fast and was competing with **S. rolfsii,** suggesting their high competitive ability compared to the test pathogen. Among the eleven rhizosphere fungi tested all the three **Trichoderma** spp. and **Aspergillus niger** only showed mycoparasitic activity in dual culture. **A. versicolor, Fusarium oxysporum** and **Penicillium oxalicum** showed mutual intermingling growth, while **A. flavus** inhibited the growth and grow over **S. rolfsii.** Release of inhibitory substances (i.e. metabolites) into the medium by **F. solani** resulted in direct inhibition of the growth of **S. rolfsii.** In most of the cases, slow growth rate of **S. rolfsii** suggests rapid utilization of nutrients by the antagonists in dual culture. Nutrient depletion, space and toxic substances (antibiotic

like substances/metabolites) produced by fungi are known to play a dominant role in antagonism and these factors usually are governed by the physico-chemical nature of the environment (Garret, 1963; Burgess and Griffin, 1967).

A clear inhibition zone was produced by **T. viride** while others did not show any inhibition zone. Attachment to **S. rolfsii** mycelium by the conidia of **T. harzianum** in the present study, suggests that the conidia absorbs nutrients from the host mycelium, as well as disintegrates the hyphal wall by the enzymes released by them (Elad **et al**, 1983a,b). This helps in penetration, growth, abosrption, lysis and bursting of host hyphae by the parasite. **T. koningii** mycelium coiled around the hypha of **S. rolfsii** while **T. viride** show coiling, absorption by conidia, lysis, penetration, bursting of hyphae etc. In some cases formation of conidia inside the host hyphae was also detected. This formation may be due to the fact that the parasitizing hyphae grew well inside the host hyphae as long as sufficient nutrient was available but at the later stages of growth due to depletion of nutrients inside the host, the parasite ceased its growth and started forming reproductive structures (i.e. conidium). The formation of resting structures by **Fusarium udum** inside the host hyphae of **Rhizopus nigricans** has also been reported by Rai **et al** (1978). Since the growth of the host hyphae is suppressed and the parasite nurishes itself from the dead host hyphae,

the parasitic behaviour as described above may be categorised as of necrotrophic type of parasitism (Barnett and Binder, 1973).

S. rolfsii was also found to parasitized **Aspergillus niger**. The coiling, penetration and growth of **S. rolfsii** mycelium inside the conidiophore of **A. niger** was observed. This type of parasitism i.e. necrotrophic type have also been observed by Arora and Dwivedi (1979). It is also an established fact that the narrower hyphae, generally penetrates the broader one (Dennis and Webster, 1971). Penetration by **S. rolfsii** to **A. niger** conidiophores strongly supports this view. Similar conclusion can also be drawn for **Trichoderma** spp.

Although **S. rolfsii** mycelium and resting structures (i.e. sclerotium) parasitized by **Trichoderma** spp. but their population in the soil was maintained probably through the penetration, multiplication and production of new sclerotia within other soil inhabitant hosts (i.e. **A. niger**) resulting in maintenance of inoculum density and perenation in the soil, which subsequently results in disease developement, as soon as the host plants (seeds/seedlings) are in the vicinity.

Care should be taken during the application of the antagonists to soil, to achieve biological control of soil-borne pathogen, as they find it difficult to establish

themselves in highly buffered soil microbiological system. This must be the reason that in most of the cases, reduction in disease severity has been obtained only under controlled condition (i.e. in green house condition), but very few reports are available on the success in the field condition.

Backman and Rodriquez-Kabana (1975) developed a system for culturing **T. harzianum** by using diatomaceous earth granule impregnated with molasses, which successfully controlled southern blight of peanut caused by **S. rolfsii**. Hadar **et al** (1979) found an isolate of **T. harzianum**, which directly attacked the mycelium of **R. solani**, when grown together. This strain, when applied as wheat bran culture into **R. solani** infested soil, effectively controlled damping off of bean, tomato and egg plants (Elad **et al**, 1980). All the isolates used in the present study were obtained from the soybean rhizosphere grown in **S. rolfsii** infested soil. Hence, when these were applied after growing them in sand wheat grain medium to soil, they (i.e. **T. harzianum, T. koningii**) could estblish themselves easily and grew profusely in the soil, resulting in reduction in inoculum potential of **S. rolfsii** and consequently, a reduction in disease severity was achieved.

The disease severity has been observed to have relationship with the amount of introduced antagonists inoculum into the soil. With the increase in inoculum, better control

of the disease was achieved. This suggests that the **Trichoderma** spp. suppressed the growth and multiplication of **S. rolfsii** in natural soil through competition and antagonism, resulting in delay of symptom expression and disease severity.

Increase in the yield of soybean plants grown in the antagonistic fungi amended soil (except **Fusarium** spp.) suggests the importance of these antagonists for the control of soybean foot rot caused by **S. rolfsii** and better yield production. Fusarium amendment may adversely affect the metabolic activity of the host plant due to the production of toxic metabolites (i.e. fusaric acid) resulting in decreased yield. **T. harzianum** not only gave good control of the disease but also gave highest yield, irrespective of the amount of inoculum used. A comparative reduction in the yield was observed in the plants grown in the higher concentration of **Trichoderma koningii** amended soil.

A conclusion may be drawn from the above discussion that the antagonistic fungi in particular live saprophytically in the soil and in rhizosphere, probably produce some antibiotic/antibiotic like substances which inhibit or suppress the growth of the pathogen due to their highly saprophytic colonization ability in soil, reducing the inoculum potential and disease severity. The parasitic nature of **Trichoderma** spp. may also have contributed in the reduction of the inoculum

potential of **S. rolfsii** causing a reduction in the disease severity. These findings further stengthen the possibility of introducing **Trichoderma harzianum** and **T. koningii** in the field soil for controlling **S. rolfsii** causing foot rot disease of soybean.

CHAPTER VIII

STUDIES ON THE EFFECT OF ORGANIC AND INORGANIC AMENDMENTS ON THE SOIL AND RHIZOSPHERE MICROFLORA OF MAIZE IN RELATION TO THE BIOLOGY AND CONTROL OF SCLEROTIUM ROLFSII

INTRODUCTION

Plant tissue either living, dead or under decomposition is a major components of the soil environment. The chemicals liberated from the tissue mixed with soil, changes the soil microflora and fauna. The liberated chemicals may be either beneficial or detrimental to successive crops depending upon the environmental conditions and upon the length of time, the residues taken for decomposition (Mc. Calla and Haskins, 1964; Patrick et al, 1964; Patrick and Toussoun, 1965).

It is known that the organic amendments affect the pathogen and other soil-borne fungi indirectly through their influence on soil microflora. Due to the presence of saprophytic microflora and the absence of living host plant, the pathogen remain suppressed in soil. During decomposition process of plant materials, a considerable amount of volatile ammonia and other chemicals are liberated from the tissue, which play a major role in the root disease initiation as well as disease control. Immature plant tissue has been found to liberate more inorganic nitrogen in soil than that of mature plant tissue (Lewis and Papavizas, 1975).

Inorganic amendments differ in several ways from that of organic amendments of plant origin. They are much simpler and applied at a lower rate than that of organic amendments. Their effect on plants are either immediate

without mediation through soil microorganism or indirect through biological transformations.

In view of the difficulty of establishing a new population of antagonistic microorganism in a natural microbial environment, the addition of amendments (organic and inorganic) to soil sometime stimulate the microflora which may be either active or antagonistic to the pathogen, seems to be a promising approach to the biological control of soil-borne plant pathogen.

Much importance has been paid to study the effect of amendments to soil in relation to disease control, but very few information is available on their effect on the rhizosphere. Therefore, an attempt was made to determine whether addition of some organic (i.e. **Eupatorium adenophorum, E. riparium**) and inorganic (i.e. Urea) amendments causes any change in the rhizosphere microflora in relation to disease development of maize (**Zea mays** L.) plants grown in **Sclerotium rolfsii** infested soil.

MATERIALS AND METHODS

Sclerotium rolfsii isolated from infected maize cob show faster growth and produced large number of small brown sclerotia. Pure culture of this isolate was made in PDA medium and was kept at 4±1°c for further work.

Amendments used

Few selected organic and inorganic amendments viz.

A. Organic amendments: Fresh green leaves of

1. **Eupatorium adenophorum** spreng.
2. **Eupatorium riparium** Regel.
3. **Helianthus annuus** Linn.
4. **Pinus kesiya** Royle ex Gordon. used in different concentrations (1, 2, 3% v/v).

B. Inorganic amendments: Lower concentrations (0.25, 0.5%w/v) of inorganic chemicals viz.

1. Calcium carbonate.
2. Calcium nitrate.
3. Ammonium nitrate.
4. Rock phosphate.
5. Zinc sulphate.
6. Urea were used in the present study against the growth and sclerotial germination of **S. rolfsii** (maize isolate) **in vitro.**

Effect on seed germination

Maize seeds were surface sterilised with 0.1% mercuric chloride solution for 1 min and was washed several times to remove the traces of mercuric chloride. Fifty seeds were soaked in the aqueous solution of different leaf extract and inorganic chemicals separately for half an hour and was kept in moist chamber and incubated for 7 days at 25±1°c. Similarly, control set was also prepared with distilled water only. Percentage germination and radicle growth was

measured after the said period of incubation.

Pathogenicity study and the method of application of amendments

The experiment on the effect of amendments on maize seedlings were carried out in **S. rolfsii** infested natural soil, at ICAR farm, Barapani (Plate 22; the area from where the infected maize cobs were collected). For preliminary observation, selected organic (i.e. **Eupatorium adenophorum** and **E. riparium**) and inorganic (i.e. urea only) amendments were applied directly to the experimental plots (12 sq.m.) in a single terrace. Different concentrations of the amendments (10, 20 q/ha for organic and 40 kg/ha for inorganic) were applied to soil atleast two weeks before sowing of the seeds.

Six rows were made in each plot (12 sq.m.) and maize seeds were sown at an appropriate spacing (i.e. 12 inches approx).

Effect of amendments on the rhizosphere

When the seedlings were about fifteen days old, ten seedlings were uprooted gently, without disturbing the root system along with adhering soil. Rhizosphere microflora and 'rhizosphere effect' was calculated following the modified method of Timonin (1940) given by Baruah and Dutta (1978) as described earlier (page 39).

Plate 22: A view of maize plants at ICAR farm Barapani, from where the infected maize cobs were collected.

PLATE-22

Population dynamics of Sclerotium rolfsii in soil

Soil samples were collected at random from the amended plots, and a composite sample for each amendment was made. The population of **S. rolfsii** was estimated on a monthly basis for four months following slightly modified selective medium as proposed by Backman and Rodriguez-Kabana (1976) described in page 42 .

Effect of amendments on plant height

Plant height was recorded fortnightly for a period of two months, till the plant produced male flower.

Effect on disease development and severity

The disease severity/disease control was calculated on the basis of the number of plants showing symptoms out of the total number of plants grown in the same treatment. The record was made upto 45 days since no increase in infection rate was observed after that period.

Effect on the yield

Yield/plant was calculated on the basis of the total maize cobs produced in each treatment/control.

RESULTS

Effect of different organic amendments on the radial growth of **S. rolfsii in vitro** revealed that, **Eupatorium**

adenophorum and **E. riparium**, slightly inhibited the growth of **S. rolfsii**. The percentage inhibition was recorded as 38.88% and 38.55% respectively at higher concentration (i.e. 3% v/v) only (Table 8.1). **Pinus kesiya** needles extract also inhibited the growth but to a lesser extent (24.4%), whereas **Helianthus annuus** did not show any effect.

Almost all the inorganic chemicals used, showed inhibition in growth of **S. rolfsii in vitro**. Higher inhibition (100%) was observed with urea (0.25% w/v) followed by zinc sulphate (61.11% at 0.5% w/v). The inhibition increased with the increase in concentration of the inorganic chemicals used (Table 8.2).

The chemicals apparently did not show any inhibitory effect on the sclerotial germination of **S. rolfsii**, excepting urea and ammonium nitrate at higher concentration (0.5% w/v), where 50% reduction in sclerotial germination was observed.

Effect on seed germination

The amendments did not show any toxic affect on the maize seed germination **in vitro**. Radicle growth was found to be reduced following the application of leaf extracts. Among the inorganic chemicals, urea and calcium nitrate showed slight toxicity while, others show stimulatory effect (Table 8.3).

TABLE 8.1 : Effect of Organic amendments on the growth (% inhibition)[1] of **S. rolfsii** (isolated from maize) **in vitro.**

Amendments	Concentration(%)	Period of incubation (hours)			
		24	48	72	96
Eupatorium adenophorum	1	63.60	37.50	13.95	20.33
	2	68.19	41.75	25.66	27.77
	3	72.79	55.75	41.84	38.88
Eupatorium riparium	1	38.86	20.75	32.63	28.88
	2	29.30	12.50	32.63	31.44
	3	35.33	25.00	38.63	38.55
Pinus kesiya	1	53.00	26.75	-	-
	2	51.40	29.25	3.34	-
	3	49.66	43.25	32.63	24.44
Helianthus annuus	1	-	-	2.37	-
	2	-	-	9.34	-
	3	-	-	13.11	-

1) Percentage of inhibition was calculated as compared to control.
(-) No inhibition

TABLE 8.2 : Effect of Inorganic amendments on the growth (% inhibition)[1] of **S. rolfsii** (isolated from maize) **in vitro.**

Amendments	Concentration(%)	Period of incubation (hours)			
		24	48	72	96
Calcium carbonate	0.25	56.63	48.25	41.84	33.33
	0.50	68.19	50.00	43.79	40.78
Calcium nitrate	0.25	62.54	46.00	46.58	40.78
	0.50	46.99	37.50	44.63	42.56
Ammonium nitrate	0.25	77.97	60.00	59.55	51.89
	0.50	63.60	50.75	55.78	53.67
Rock phosphate	0.25	29.32	8.25	39.60	38.89
	0.50	40.98	23.25	41.00	46.33
Zinc sulphate	0.25	43.46	37.50	47.42	44.44
	0.50	82.33	64.25	67.50	61.11
Urea	0.25	100	100	100	100

1) Percentage of inhibition was calculated as compared to control.

TABLE 8.3 : Effect of soil amendments on the maize seed germination[1] and radicle growth[2] **in vitro.**

Amendments	Concentration (%)	Seed germination (%)	Radicle growth (mm)
ORGANIC			
Eupatorium adenophorum	1.00	100	32.60±1.17
	2.00	100	29.90±1.19
	3.00	100	26.80±1.07
Eupatorium riparium	1.00	100	30.40±1.28
	2.00	100	36.20±1.23
	3.00	100	34.20±1.06
Helianthus annuus	1.00	100	33.40±1.36
	2.00	100	33.50±1.30
	3.00	100	37.70±1.19
Pinus kesiya	1.00	100	35.75±1.23
	2.00	100	28.38±1.40
	3.00	100	31.90±1.00
Control (without amendment)	-	100	49.70±1.51
INORGANIC			
Calcium carbonate	0.25	100	43.60±0.34
	0.50	100	54.10±0.36
Calcium nitrate	0.25	100	40.00±0.43
	0.50	100	30.06±0.44
Urea	0.25	100	43.00±0.38
	0.50	100	33.00±0.28
Ammonium nitrate	0.25	100	72.00±0.37
	0.50	100	57.50±0.80
Rock phosphate	0.25	100	83.70±0.80
	0.50	100	71.13±1.12
Control (without amendment)	-	100	44.30±0.30

1) Calculations based on 30 seeds in each case.
2) Mean of 10 replicates with ±S.E.

Effect of soil amendments on the rhizosphere microflora

Soil amendment changed the microbial population in the rhizosphere considerably. It was observed that the fungal and bacterial population decreased, while actinomycete found to have stimulated in the rhizosphere following organic amendments. **E. adenophorum** was found to be toxic as it directly affected the rhizosphere microflora compared to **E. riparium** amendment. Soil amended with urea also reduced rhizosphere microflora (Table 8.4).

Effect on the particular genera of fungi

It can be seen from the table 8.5 that soil amendments affected the particular genera of fungi in the maize rhizosphere. All the amendments increased **Penicillium** spp. population. **E. riparium** (10, 20 q/ha) increased **Trichoderma harzianum** and Aspergilli while urea increased Aspergilli mostly in the rhizosphere. Altogether twenty eight species belonging to twenty fungal genera and some unidentified fungi imperfecti were isolated from the rhizosphere.

Effect of soil amendments on disease development and severity

The infection increased slowly upto 45 days of plant age after which no further increase was recorded. The percentage of infection reduced considerably compared to non amended infected control. **Eupatorium adenophorum** (20 q/ha) amendment

TABLE 8.4 : Effect of soil amendments on the rhizosphere microflora of maize.

Amendments	Concentration (q/ha)	Total population in thousands g^{-1} dry soil		
		Fungi (10^4)	Actinomycete (2×10^4)	Bacteria (3×10^4)
Eupatorium adenophorum	10.0	7.92	265.40	320.00
	20.0	6.70	308.30	350.80
Eupatorium riparium	10.0	10.40	345.80	373.30
	20.0	7.50	387.08	320.80
Urea	0.4	9.58	165.00	302.50
Infected control (without amendments)	-	11.25	263.30	425.80

TABLE 8.5 : Effect of soil amendments (Organic and Inorganic) on the percentage of relative abundance of rhizosphere fungi.

Rhizosphere fungi	Infected control (without amendment)	Eupatorium adenophorum (A)	Eupatorium adenophorum (B)	Eupatorium riparium (A)	Eupatorium riparium (B)	Urea (C)
Absidia sp.	7.40	-	-	-	-	4.30
Acremonium sp.	-	-	-	-	5.00	-
Aspergillus candidus Link ex.Fries.	-	-	-	-	5.00	4.30
A. flavus Link. ex. Fries.	-	-	-	-	-	8.60
A. niger van Tieghem	11.10	-	9.52	-	10.00	-
A. versicolor (vuillemin) Tiraboschi.	3.70	10.50	-	-	-	4.30
Bipolaris maydis (Nisikado) Shoemaker.	3.70	-	-	-	10.00	-
Cephalosporium roseo-griseum Saksena.	11.10	10.50	4.76	4.00	-	4.30
Cladosporium sp.	-	-	-	8.00	5.00	-
Cunnighamella sp.	-	-	-	4.00	-	-
Curvularia lunata (Wakker) Boedijn.	-	15.78	-	-	-	-
Fusarium oxysporum	-	5.30	-	-	-	4.30
Fusarium sp.	3.70	5.30	9.52	16.00	-	-
Geotrichum sp.	7.40	10.50	9.52	4.00	5.00	-
Helminthosporium sp.	-	-	-	4.00	-	-
Humicola sp.	-	5.30	-	-	5.00	-
Mucor plumbeus Bonorden.	14.81	5.30	9.52	8.00	10.00	13.00
Penicillium chrysogenum Thom.	3.70	-	4.76	12.00	-	8.60
P. rubrum Stoll.	-	10.50	14.28	-	-	4.30
P. vermiculatum Dangeard.	-	-	-	-	5.00	-
Pythiopsis sp.	-	-	-	4.00	-	8.60
Pythium sp.	-	-	-	4.00	-	-
Rhizopus stolonifer (Ehrenb ex. Link) Lind 1913.	-	-	9.52	4.00	-	8.60
Trichoderma harzianum Rifai.	-	-	4.76	20.00	25.00	-
T. koningii oudemans.	3.70	-	-	-	-	8.60
T. viride Pers. ex. Fries.	3.70	5.20	-	-	-	-
Verticillium sp.	-	-	-	-	5.00	-
Others genera of fungi imperfecti.	-	-	9.52	4.00	-	-
Yeast	25.90	15.80	14.28	4.00	10.00	17.39

A=10 q/ha; B=20 q/ha; C=40kg/ha. Total number of genera = 20; Total no. of species=28; Total no. of unidentified genera = 3.

produced higher reduction in disease severity (2.8% infection) followed by **E. riparium** (4.8%). More than 50% reduction in infection, was observed almost in all the cases (Table 8.6).

Effect of soil amendments on the population dynamics of Sclerotium rolfsii in soil

All the amendments used were found to increase the population of **S. rolfsii** in soil at the initial stage. The population gradually declined in case of **E. riparium** amendment. Although **E. adenophorum** amendment to soil, gradually decreased the population upto three months, but subsequently the population increased. No remarkable change in the population of **S. rolfsii** due to urea amendment was observed during the four months of investigation (Table 8.7). In non amended control the population was found to increase considerably.

Effect of soil amendments on the plant height

The plant height was recorded for two months (upto flowering period) only. **E. adenophorum** (20 q/ha) and urea (40 kg/ha) reduced the height slightly, while **E. riparium** showed an increase over non amended infected control (Table 8.8; Plate 23, 24).

Effect of soil amendments on yield

Amendments did not show any effect on the yield. A minimum of two healthy maize cobs were found to be produced

TABLE 8.6 : Effect of soil amendments on the disease development (%)[1] in Maize.

Amendments	Concentration (q/ha)	Sampling period (days)		
		15	30	45
Eupatorium adenophorum	10.0	2.27	3.98	5.10
	20.0	1.19	1.98	2.80
Eupatorium riparium	10.0	3.28	6.10	6.60
	20.0	2.16	4.30	4.80
Urea	0.4	2.82	6.80	7.90
Infected control (without amendments)	-	3.30	7.22	13.90

1) Calculation based from the plants grown in 12 sq.m plot.

TABLE 8.7 : Population dynamics ($x10^4$ g^{-1} dry soil) of **S. rolfsii** in amended soil.

Amendments	Concentrations (q/ha)	Sampling period (months)			
		1	2	3	4
Eupatorium adenophorum	10.0	5.29	3.70	1.70	4.80
	20.0	3.45	3.41	1.58	4.20
Eupatorium riparium	10.0	4.50	3.58	2.38	1.90
	20.0	4.90	3.20	2.54	1.50
Urea	0.4	4.16	4.08	4.67	4.83
Infected control (without amendments)	-	2.80	6.07	[illegible]	[illegible]

TABLE 8.8 : Effect of soil amendments on the height of Maize Plant (Mean ± S.E.)[1].

Amendments	Concentration (g/ha)	Time in weeks			
		2	4	6	8
Eupatorium adenophorum	10.0	10.5±0.21	31.3±2.83	77.0±1.26	158.0±5.74
	20.0	9.3±0.18	29.1±0.95	77.0±0.25	140.9±7.78
Eupatorium riparium	10.0	9.2±0.17	33.9±1.7	72.0±0.85	158.4±6.60
	20.0	9.3±0.20	27.7±1.30	76.0±0.18	173.2±4.22
Urea	0.4	9.5±0.24	30.2±2.75	80.4±1.28	149.1±4.38
Infected control (without amendments)	-	8.5±0.13	22.5±2.12	74.0±0.78	157.3±5.28

1) Calculations based on ten replicates.

Plate 23: Showing the maize plants grown in (i) infested soil and in the (ii) infested soil amended with **Eupatorium riparium.**

PLATE-23

(i)

(ii)

Plate 24: Showing the reduced growth of maize plants following (i) **Eupatorium adenophorum** and (ii) Urea amendments to soil.

PLATE - 24

(i)

(ii)

by each plant.

DISCUSSION

The maize isolate of **S. rolfsii** usually grow faster and produced large number of small brown sclerotia compared to the isolate from soybean, which produced less number of large brown sclerotia. This isolate was found to be slightly inhibited (38% approx.) by the leaf extracts of **E. adenophorum** and **E. riparium**, while **Pinus kesiya** needle show 24.4% inhibition. This suggests that the inhibitory compounds in the leaves, have effect on the growth of **S. rolfsii**, irrespective of the isolates used, as evidenced from the result presented in the previous chapters for soybean. The growth of this isolate was also found to be completely inhibited by urea amendment, similar to the result obtained for **S. rolfsii** isolate of soybean.

The organic amendments reduced the fungal and bacterial population in the rhizosphere. Increase in the population of **Penicillium** spp., **Aspergillus** spp. and **Trichoderma** spp. in the rhizosphere was also observed. It was reported earlier that the litter of **E. adenophorum** and **E. riparium** release inhibitory substances during their decomposition process, which affects the soil microorganisms (Rai and Tripathi, 1984). Decrease in microbial population in the rhizosphere due to organic soil amendments in the present study supports

their view. It was also mentioned earlier by the author that an increase in **Penicillium** spp., **Trichoderma** spp. and **Aspergillus** spp. population in the rhizosphere following amendments, causing a reduction in the disease severity of soybean caused by **S. rolfsii** possibly by their antagonistic activities in soil/rhizosphere. Reports on the selective increase of **Trichoderma** spp. population after **E. riparium** amendment (Rai and Tripathi, 1984), **Penicillium** spp. (Reddy, 1959) and **Aspergillus** spp. (Agnihotri, 1964) in soil due to urea application, conforms with the present findings.

Urea amendment to soil reduced the rhizosphere microflora. Similar result was also reported earlier (Dutta and Deb, 1986), where a significant reduction in the rhizosphere microflora of soybean following urea (0.25%) and rock phosphate (0.5%) amendments to soil was observed.

The soil amendments reduced the disease severity. This reduction might have been caused by the individual or combined effort of each of the living components involved in the disease viz. the pathogen, the host and the rhizosphere microflora. It is known that the amendments may affect disease severity through their effect on the germination of the pathogen's propagule or mycelial growth in soil or on the host surface and hence on the colonisation of the host tissue. In the present study, increase in **S. rolfsii** population

following organic amendments is possibly due to the break down of the exogenous dormancy and due to the saprophytic colonisation on the organic substrates that amended to the soil. Subsequently a decline in their population, may be due to the slow release of inhibitory substances to the soil, during the decomposition process. The increase in **Trichoderma** spp. and **Penicillium** spp. population in the soil and rhizosphere might have created an antagonistic environment for the pathogen in the soil, consequently, a reduction in disease severity occurred.

Urea amendment to soil, increased the **S. rolfsii** population and the population was found to be static, throughout the sampling period, which suggests that the concentration of urea used (40 kg/ha) was not too toxic to **S. rolfsii** in the soil resulting a steady increase in disease severity compared to other organic amendments.

Maize cob production was not affected by soil amendments. Interestingly, it was found that the cobs collected from the plants grown in amended soil were healthy and not infected by **S. rolfsii**. Hence, a conclusion can be made from the present study that although the experimental soil was highly infested by **S. rolfsii**, only a low percentage of seedling was found to be infected in the field. The infection in the cobs appear might be due to the contamination with

sclerotia of **S. rolfsii** while storage. Hence, proper care should be taken during storage. Further experimentation is required for a detailed study of this isolate obtained from maize cobs.

GENERAL DISCUSSION

It is known that the fungal propagules of soil-borne plant pathogens remain resting in soil either because of inherent dormancy of fungistasis, a wide spread natural soil toxicity manifested as non specific inhibition of propagules germination (Lockwood, 1977). In the present work, it has been observed that the **Sclerotium rolfsii** is sensitive to soil fungistasis and the sclerotial germination varied with the season i.e. less in summer months and more in winter. Studies on the soil fungistasis revealed that the factor is seasonally variable i.e. more in summer months than in winter. No correlation was observed between the seasonal variation and of microbial population (excepting with fungi in grassland and with actinomycete in garden), but a direct relationship was observed with available nutrients (i.e. total sugar) in the soil. Correlation also exist between the soil microbial activity (i.e. dehydrogenase) and soil fungistasis. Present study on soil fungistasis generated informations which could be utilised in the control of foot rot disease caused by **S. rolfsii**. It has been observed that certain organic and inorganic amendments stimulated the sclerotial germination process suggesting that if these soil amendments are applied much earlier to the sowing of soybean, it could break the "imposed dormancy" (i.e. due to soil fungistasis) of the sclerotium of **S. rolfsii** in soil and will allow a germination-lysis to the sclerotium and also a reduction in inoculum potential resulting in a reduction of disease severity to the soybean crop.

The application of organic (i.e. **Eupatorium adenophorum, E. riparium, Pinus kesiya** and **Helianthus annuus**) and inorganic (i.e. calcium carbonate, calcium nitrate, urea, rock phosphate, zinc sulphate and ammonium nitrate) amendment to soil was found to be potentially effective. Increase in the rhizosphere microflora and the increased antagonistic population in the rhizosphere being helpful in reducing the inoculum potential or even in eliminating the pathogen (i.e. **S. rolfsii**) from the root region. Since some of these amendments (i.e. **E. adenophorum, E. riparium,** urea, $ZnSO_4$) were found to influence the pathogen directly **in vitro**, they are likely to have similar effect in the field. Some of them also increase the population of soil microflora, thereby reducing the saprophytic competition by the pathogen. The mechanism of infection and control seemed to relate to a triangular problem involving plant, pathogen and rhizosphere microflora. Thus, further work is necessary to determine the effect of organic and inorganic chemicals upon the host, pathogen and other microorganisms in the rhizosphere.

The application of chemicals (i.e. nitrofurans, antibiotics, fungicides) to the foliage/soil drench in relation to the control of foot rot of soybean caused by **S. rolfsii** was found to be successful. These treatments also affected the rhizosphere microorganisms and influenced the growth of particular genera of fungi. These factors may have been instrumental in causing

a reduction in disease severity. It seems quite logical that the higher level of microbial activity, due to foliar spray/ soil drench by chemicals, not surprisingly gave rise to antagonistic and associative interactions between certain groups of microorganisms and the pathogen in the rhizosphere of the soybean plant grown in the **S. rolfsii** infested soil. Consequently there was a reduction in the inoculum potential of the pathogen followed by a reduction in disease severity.

It was further observed that the application of chemicals by foliar spray/soil drench to some extent controlled the disease in the soybean plant by delaying the onset of disease, and reducing the percentage of infection. Experimental evidence indicates that there is not necessarily a direct relationship between fungitoxicity and chemotherapeutic potency, since, even when the compounds were not fungitoxic, they were active as chemotherapeutants. The reduction of disease severity by the non fungitoxic chemicals seemed to be due to an altered host metabolism. Thus, some of the treatments which reduced disease severity in the **S. rolfsii** infected soybean plants, also increased the production of a better yield compared to controls. Davis and Dimond (1952) also noted a direct correlation between the chemotherapeutic activity of the synthetic organic chemicals and their capacity to alter the host metabolism. They observed a change in gross morphology and in the concentration of certain biochemical constituents of the host (i.e.

increased concentration of reducing sugar and a number of water soluble nitrogen fractions). Dimond (1963) suggested that a chemotherapeutic compound must be inside the plant and must have the following capacities (a) to kill the pathogen as it enter the host (b) to rid the host of an established infection or (c) to increase host resistance. Most of the chemicals used in the present investigation have fulfilled one or more of these.

The phenomenon of antagonism between the normal soil microflora and root infecting pathogens has received much attention during recent years because of the possibility of finding a method giving a high degree of biological control. Therefore, attempts to increase antagonistic microorganisms in the rhizosphere by the application of organic and inorganic materials to soil or by foliar sprays of selective chemicals seemed to be a very useful approach to control soil-borne plant disease. In the present work, most of the antagonistic fungi isolated from the soybean rhizosphere did show a wide range of inhibiting effect on **S. rolfsii in vitro.** Among the **Trichoderma** spp., **T. harzianum** and **T. koningii** were found to be most efficient antagonists, reducing the disease severity of soybean when amended to the **S. rolfsii** infested soil. The highest disease control was obtained with **T. harzianum** amendment. **T. koningii** also showed reduction in disease severity in a similar manner. An increase in the reduction of disease with an increase in concentration of the antagonist inoculum was

also recorded. The population of **S. rolfsii** in antagonist amended soil decreased considerably compared to the non amended infested control, which can be correlated to the control of the disease acheived with such treatments (i.e. **T. harzianum**). An increased yield of soybean has also been recorded with antagonist treatment, which was found to be interesting and warrents further attention.

Attempt to manipulate antagonism especially mycoparasitism, have been found to be successful. Mycoparasitism is largely unexplored and poorly understood in relation to survival of fungal propagules in soil. Further study on the ecology of mycoparasitism is required to fully understand the biological control of plant diseases, which has been achieved in the present work. The present findings corroborates with the findings of Wells **et al** (1972) and Hadar **et al** (1979) where they acheived significant reduction of disease caused by **S. rolfsii** and **R. solani** respectively.

Understanding the ecological factors affecting the distribution of antagonists (i.e. **Trichoderma** spp.) in their natural habitats may lead to our understanding of the population dynamics of the antagonist, its survival and proliferation in soil and in the rhizosphere of host plants may add to our knowledge of their action in soil ecosystem and control of soil-borne diseases.

SUMMARY

In the modern system of soil-borne disease management, control with the help of soil amendments is comparatively a new approach. The soil amendments may reduce the disease severity either by inhibiting the growth of the pathogen or by stimulating the antagonistic soil and rhizosphere microflora. The fungal propagules are known to remain dormant in soil, which may germinate due to break of "imposed dormancy" (i.e. due to soil fungistasis) following soil amendments and may increase or decrease the disease severity. Therefore, it is important to develop methods, to reduce the number of propagules of the pathogen in soil below a dynamic damage threshold level. In the present investigation, "Studies on the effect of organic and inorganic amendments on the soil and rhizosphere microflora in relation to the biology and control of soil-borne plant pathogen (**Sclerotium rolfsii** Sacc.)", emphasis has been given to control the pathogen (i.e. **S. rolfsii**) with the help of some easily available green plant materials and with some organic/inorganic chemicals (i.e. inorganic fertilisers, antibiotics and fungicides).

The thesis is presented under following headings:

General Introduction, Review of Literature, Environmental features with General materials and methods, Experimentals (eight chapters), General Discussion, Summary and References. Each chapter has an Introduction, Materials and Methods, Results and Discussion. The experimental work has been divided

into two parts. Part one "Soil Survival" with one chapter i.e. Studies on soil fungistasis, while Part two "Plant soil relationship", consists of seven chapters viz. (1) Effect of organic, (2) inorganic amendments on the soil, rhizosphere microflora, disease development and yield, (3) effect of nitrofurans, (4) antibiotics, (5) fungicides on the rhizosphere microflora, disease development and yield, (6) studies on antagonism and biological control of **S. rolfsii** and (7) studies on the effect of organic and inorganic amendments to soil on rhizosphere microflora and disease development in maize.

Part - I: Soil fungistatic activity against **S. rolfsii** was studied in relation to seasonal variation, microbial population, available nutrients and microbial activity (i.e. dehydrogenase) in three different soil types (i.e. forest, garden and grassland soil). It has been observed that soil fungistasis is seasonally variable i.e. with an increase in summer months compared to the winter. No correlation was observed between the seasonal variation of the factor and of microbial population in the soil in general, but a direct correlation with available nutrients (i.e. total sugar) in soil has been observed. Among the physico-chemical factors, soil moisture and enzyme activity shows a direct relationship with the seasonal variation of soil fungistasis. Ethrel (2 chloro ethane phosphonic acid), an ethylene generator in aqueous solution, was found to induce fungistasis in sterilised soil which was otherwise non fungi-

static. It inhibits the sclerotial/spore germination in soil, in aqueous solution and affect soil microbes even at lower concentration (1 µl/L). The inhibition due to ethrel was reduced by supplementing glucose (1% and 10%) in experimental soil. This supports the inhibitor and stimulator theory to balance soil fungistasis proposed by Smith (1973). The probable pathway and the factors involved in soil fungistasis has been discussed.

Part - II: Among the organic amendments, **Eupatorium adenophorum** followed by **E. riparium** leaf extract, inhibited the growth of **S. rolfsii in vitro**. The inhibition in sclerotial germination is positively correlated with the concentration of the plant extracts used. Viability of the sclerotium in soil decreased (excepting **E. adenophorum**) with the increase in concentration of the amendments applied and with time. Organic amendments did not have any adverse affect on soybean seed germination. Amendments significantly increases total phenolic compounds in the treated soybean radicles **in vitro**.

All the soil amendments stimulated fungi, actinomycete and bacteria (excepting **E. adenophorum** and **E. riparium**) in soil, whereas, a significant increase in bacterial population was observed in soybean rhizosphere. Aspergilli, mucorales, **Trichoderma** spp. were found to be stimulated in soil and rhizosphere. A comparatively higher population of Aspergilli was observed after the application of **Helianthus annuus** and **Pinus**

kesiya amendment to soil and **E. adenophorum** (3% w/w), amendment in rhizosphere. **Trichoderma harzianum** and **T. koningii** were found to be stimulated in the soil and soybean rhizosphere following **E. riparium** soil amendment. A total of thirty eight and thirty two species belonging to eighteen and fourteen genera have been isolated from soil and rhizosphere, respectively.

Soil amendments (i.e. **P. kesiya, H. annuus,** Poultry litter) initially increased **S. rolfsii** population in soil, which subsequently declined. While **E. adenophorum** amendment to soil reduced the population. Although, pre-emergence seed rot was observed, but reduction in disease severity was achieved with all the soil amendments. Maximum reduction in disease severity was observed with **E. riparium** amendment.

Organic amendments to soil in general did not produce any adverse affect on the growth of soybean plants. Infact, **H. annuus** and Poultry litter amendment increased the growth significantly. All the amendments increased the dry weight of shoot, pod and yield in soybean plants. Comparatively higher yield was recorded with **E. riparium** (3%w/w) and Poultry litter (2%w/w) amendments. Under field condition, higher concentration (40q/ha) of the soil amendments reduced foot rot disease together with increased soybean yield.

Among the inorganic amendments, urea and zinc sulphate (0.25, 0.5, 1.0%w/w) suppressed the growth and sclerotial

germination of the pathogen **in vitro**. The survibality of sclerotium in soil decreased with time and concentration of the chemicals. Higher concentration of inorganic amendments decreased soybean seed germination **in vitro**. Significant increase in phenolic compounds was observed in the treated soybean radicles. Soil fungal population increased due to rock phosphate and ammonium nitrate amendment to soil. But rock phosphate (0.1, 0.25%w/w) only increased the fungal population in soybean rhizosphere. A significant increase in bacterial population in soil and rhizosphere was recorded with all the inorganic amendments used. Although, increased actinomycete population was observed in soil but the population decreased in the rhizosphere. Soil amendments stimulated mucorales in soil and mucorales and Aspergilli in the rhizosphere. Zinc sulphate (0.25%w/w) boosted Aspergilli in soil, whereas, rock phosphate (0.25%w/w) and urea (0.5%w/w) increased the same in the rhizosphere. Calcium nitrate (0.25%w/w) and zinc sulphate (0.1%w/w) stimulated penicillia in soil and rhizosphere of soybean seedlings respectively. A total of twenty seven and thirty one species belonging to seventeen and fifteen genera have been identified and isolated from the amended soil and rhizosphere respectively.

Amendments initially increased the pathogen population resulting an increase in pre-emergence seed rot. Significant increase in **S. rolfsii** population after urea amendment is probably, due to the breaking of "imposed exogenous dormancy"

by soil fungistatic factor present in the soil. The seedlings which escaped rot, delayed symptom expression by two weeks (0.5%w/w concentration in all the cases) compared to infected control. Reduction in disease severity (i.e. 30-50%) was observed with all the inorganic fertilisers used.

Higher concentration (0.5% w/w) of most of the inorganic fertilisers were found to have toxic effect on the seedling growth (i.e. height) and yield excepting calcium nitrate and urea (0.1%w/w). Urea (0.1%w/w), calcium nitrate (0.25%w/w) and calcium carbonate (0.1, 0.25%w/w) increased the shoot weight, whereas, urea (all the concentrations) increased root weight. No significant increase in soybean yield due to the inorganic soil amendments was observed.

Under field condition, a direct correlation was observed between the **S. rolfsii** population in soil and soybean foot rot in case of urea (80 kg/ha) at the initial stage. Although, zinc sulphate (5, 10kg/ha) initially increased the population but it declined slightly with time. Whereas, rock phosphate (40kg/ha) showed a gradual increase in pathogen's population. A significant reduction in disease severity was recorded with all the inorganic chemicals used, of which zinc sulphate gave the best result. Urea (80kg/ha) and zinc sulphate (10kg/ha) stimulated the plant growth which was reflected on the increase in plant height, vigour and yield as compared to infected control and the lower concentration of the chemicals.

Among the three nitrofurans tested, furazolidone (at all concentrations) and nitrofurantoin (higher concentration only), completely inhibited the growth of **S. rolfsii in vitro**. Decreased sclerotial germination was observed with the increase in concentration of the chemicals. Complete loss of viability after 30 days of incubation was observed due to furazolidone (1000 µg/L) amendment to soil. Others also reduced the number of viable sclerotia with the increase in incubation period. Significant reduction in fungal and bacterial population in the soybean rhizosphere following the foliar application of nitrofurans was observed. All the nitrofurans reduced the disease severity (more than 50%). Better yield production was achieved with furazolidone treatment only.

Antibiotic viz. actidione and thiolutin completely inhibited the growth of **S. rolfsii in vitro** even at lower concentration (20 µg/L). Others showed slight inhibition. Loss in viability of sclerotium by 50% after 15 days and complete loss after 60 days of incubation in treated soil was observed with higher concentration of all the antibiotics tested.

The concentrations of antibiotics used here, however, found to be toxic to soybean seed germination, excepting few cases i.e. thiolutin (upto 80 µg/L), streptomycin (20, 40 µg/L) and penicillin (20 µg/L), whereas, streptomycin (all the concentrations), penicillin and chloramphenicol (20, 40 µg/L) stimulated the radicle growth.

A significant decrease of fungi, bacteria and increase in actinomycete population was recorded in the rhizosphere of soybean seedlings following foliar spray with antibiotics. A total of twenty nine species belonging to thirteen genera were isolated from the rhizosphere.

Higher concentration of all the antibiotics delayed the symptom expression. Actidione followed by penicillin and thiolutin gave better control of disease (at 100 µg/L) compared to others. Slight increase in yield was recorded with thiolutin, chloramphenicol (lower concentration), followed by streptomycin (all the concentrations).

Among the seven fungicides tested, PCNB and agrosan produce highest inhibition in growth of **S. rolfsii**. Viability of sclerotium reduced when dipped in aqueous solution of sulfex and agrosan, but in soil, dicloran and agallol was found to be the most effective. Sulfex, was found to be toxic to soybean seed and seedlings, while others produced slight inhibition in seed germination only. Soil drench with fungicides, decreased the microbial population (i.e. fungi, actinomycete and bacteria) in the rhizosphere. A total of twenty nine fungal species belonging to thirteen genera have been isolated, of which Aspergilli and mucorales were found to be the dominant.

A delayed disease development was achieved in soybean plants grown in the infested soil drenched with PCNB and agallol. All the fungicides used have controlled the disease severity,

however, the best result was obtained with PCNB and agallol. Increase in yield was observed with PCNB and agrosan, irrespective of the concentrations used, while others also increased the yield but only at lower concentration. On the other hand delan and sulfex reduced the yield considerably.

In biological control studies, eleven dominant rhizosphere fungi of soybean were selected to determine their activity against the growth of **S. rolfsii in vitro** and **in vivo. Trichoderma viride** and **T. koningii** showed volatile, while **T. viride, T. koningii, T. harzianum** and **Aspergillus flavus** showed non volatile antibiotic activity resulting inhibition in growth of **S. rolfsii.** The inhibition rate decreased with the increase in incubation period. These three **Trichoderma** spp. also parasitized **S. rolfsii** mycelium through coiling, lysis, penetration, growth, conidia formation and ultimately bursting the host hyphae. A necrotrophic mycoparasitic activity of **S. rolfsii** on **Aspergillus niger** was also observed. Others show mutual intermingling growth **in vitro. T. viride** and **F. solani,** also produced inhibition zone (type C) in some cases.

Trichoderma harzianum and **T. koningii** amendment to **S. rolfsii** infested soil gave the best control of soybean foot rot disease. Population of **S. rolfsii** decreased considerably when the antagonist cultures were (i.e. **Trichoderma** spp.) separately amended to soil, which could be correlated with the decrease in disease severity. Higher yield of soybean was recorded with **Trichoderma** spp. compared to others.

Sclerotium rolfsii isolated from infected maize (**Zea mays** L) cobs differ morphologically from the soybean isolate. The isolate grew very fast producing large number of small brown sclerotia. **E. adenophorum** and **E. riparium** soil amendments reduced the **S. rolfsii** population, while urea had virtually no effect. All the soil amendments used reduced the disease severity in maize plant.

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APPENDIX

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