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

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

To

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JANUARY, 1987

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.