STUDIES ON *SCLEROTIUM RQLFSII* SACC. ON CROP PLANTS IN NORTHERN NIGERIA WITH SPECIAL EMPHASIS ON TOMATO

By

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DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented before in any previous application for a higher degree.

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DEDICATION

To my late father, catechist TIMOTHY A.R. WOKOCHA, who strove to give me education.

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ABSTRACT

Field survey conducted over a period of five years (1976 - 1981) in parts of northern Nigeria during this study, indicate that crops such as tomato (Lycopersicon esculentum), cabbage (Brassica oleraceae) and pumpkin (Cugurbita moschata) were attacked in the field by Sclerotium rolfsii, while others such as onion (Allium cepa) bulbs and potato (Solanum tuberosum) tubers were attacked in storage. Results of field surveys suggest that tomato plant was frequently attacked by the pathogen in the field.

Artificial inoculation in the greenhouse showed that all 35 tomato cultivars evaluated were susceptible to infection by S. rolfsii. Of the 40 different plant species screened for susceptibility to infection by the pathogen, only 'bahama' grass (Cynodon dactylon) appeared to be resistant to infection by S. rolfsii. Results of field surveys and artificial inoculations in the greenhouse appear to have shown for the first time in Nigeria that cabbage (B. oleraceae), spinach (Amaranthus sp.), sorghum (Sorghum vulgare) and millet (Penniseturn typhoides) are attacked by the basal stem rot disease.

Results of in vitro screening of 26 fungicides against S. rolfsii revealed that 13 of these fungicides, notable the oxanthiin compound - Vitavax-, Du-Ter 50, PCNB, Verdasan, Fernasan D and Cerosan practically prevented any mycelial growth at active ingredient concentrations as low as 5 to 50 p.p.m.

In vivo greenhouse tests showed that of the fungicides which strongly inhibited the mycelial growth of S. rolfsii on PDA, seven (Aatopam-N, captan, Dithane M-45, Aldrex T, PCNB, Calixin M and captafol) appeared to be non-phytotoxic to young tomato plants at the concentrations inhibiting mycelial growth.

An evaluation of the various factors which influence the development of the basal stem rot disease in the greenhouse revealed that pre—inoculation mechanical injury on tomato plants as well as low relative humidity significantly enhanced disease severity. Trichoderma viride completely prevented the appearance of the disease when it was inoculated on tomato plants on the same day as, or three days before inoculation with S. rolf sii, but was ineffective when inoculated three days after inoculation with the pathogen. PCNB was also found to be efficacious in reducing the basal stem rot disease when applied to soil 10 days before inoculation in the greenhouse.

In field trials conducted in Samaru to evaluate the efficacy of of some fungicides, antagonistic micro-organisms and animal manures in reducing the basal stem rot disease on tomato, PCNB and T. viride were found to be highly effective in reducing the disease.

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CHAPTER 1

INTRODUCTION

The cultivated tomato (Lycopersicon esculentum Mill.) is a weak-stemmed, warm season, herbaceous plant. Tomato is now an important ingredient in the Nigerian diet, and the demand for the processed product has increased several times in the past few years.

The Northern Guinea and Sudan Savanna Zones of Nigeria lying mainly between 9° and 12°N, possess favourable climatic and soil conditions necessary for tomato cultivation (Shivdasani, 1975). Some state governments like those in Kaduna and Kano and commercial companies, notably Cadbury, have therefore set up tomato processing industries in various parts of the country to make a locally acceptable paste. The establishment of a net-work of dams and irrigation projects for round-the-year cultivation have also greatly enhanced the production of tomatoes in these areas which have consequently become the main centres of tomato production in Nigeria. Zaria (in the Northern Guinea Savanna) and Kano (in the Sudan Savanna), together with their environs produce on the average 20 tons of marketable tomato fruits per hectare annually (Simons and Sobulo, 1975).

However, the tomato crop is highly susceptible to a large number of diseases caused by fungi and other groups of pathogens. These diseases lead to heavy losses in yield, especially in the developing countries of the tropics like Nigeria where high temperatures and relative humidity as well as inadequate facilities for disease control combine to provide ideal conditions for attack by plant pathogens.

Some of the major fungal diseases of tomato in northern Nigeria include: Early blight, caused by Alternaria solani (Ell. and Mart.)(L.R. Jones and Grout); Septoria leaf spots, incited by Septoria lycopersici Speg.; Late blight, caused by Phytophthora infestans (Mont.) DBY.; Fusarium wilt, incited by Fusarium oxysporum f.sp. lycopersici (Sacc.) Snyd.; Leaf mould induced by Cladosporium fulvum Cooke and the basal stem rot disease, incited by Sclerotium rolfsii Sacc. (Quinn, 1971, 1975; Huntington et al., 1976; Erinle, 1979).

The basal stem rot disease counts among the most destructive diseases of tomato in northern Nigeria. For example, recent investigations (Quinn, 1978 personal communication) showed that a large percentage of the tomato crop grown in the Zaria area was destroyed by S. rolfsii during the 1978 dry season. A number of workers (Quinn, 1971, 1975; Erinle, 1977) have also cited the basal stem rot disease as a major disease of tomatoes

and other vegetable crops in northern Nigeria.

Despite its importance in the northern parts of the country, the basal stem rot disease and its control have not been adequately investigated in these areas. The purpose of this study was therefore to:

(i) Conduct field surveys in different parts of northern Nigeria, particularly within the Northern Guinea and Sudan Savanna zones, in order to determine the importance of the basal stem rot disease on tomato and other crops.

(ii) Investigate aspects of the pathogenicity of the incitant, S. rolfsii, on tomato and several other crops and

(iii) Evaluate various practices for the control of the disease with the aim of recommending suitable control measures.

CHAPTER 2

2.0 REVIEW OF LITERATURE

2.1 Name of the disease

This disease and its incitant have been known by various names (synonyms). For the disease such names include: "Stem rot", "Southern blight", "Sclerotium wilt", "Southern stem rot", "Sclerotium blight" (Garren, 1959); "Sclerotium base rot" (Aberdeen, 1976) and "Basal stem rot" (Oladiran and Okusanya, 1980; Odunfa, 1981). In the present investigation, the name "basal stem rot" is used to describe the disease. This name appears to be more descriptive of the mode of attack of the pathogen, and the major symptom of the disease.

2.2 The causal organism

Sclerotium rolfsii was first described and reported on tomato by Peter Henry Rolfs in Florida in 1892 (Aycock, 1966). Later in the same year, the pathogen was isolated and its pathogenicity on tomato proved (Epps et al., 1951). In 1911, Saccardo gave the fungus its present name (Aycock, 1966).

2.2.1 The perfect stage of the fungus.

Sclerotium rolfsii has a basidiomycete perfect stage which occurs only rarely in nature (West, 1947;

Goto, 1952), but which according to Hopkins (1956) has not yet been observed in Africa. However, there are reports of the formation of the basidial stage of the fungus on agar media in laboratory cultures (Goto, 1930; Misra and Hague, 1960; Punja et al., 1982). This perfect stage of the fungus was named Corticium rolfsii Sacc. (Curzi, 1931) and Pellicularia rolfsii (Sacc.) West (Wheeler, 1976). Talbot (1973) suggested that the basidial stage of S. rolfsii belonged to the genus Athelia, and based on this suggestion, Tu and Kimbrough (1978) made the combination Athelia rolfsii (Curzi) Tu and Kimbrough.

2.2.2 Fungal hyphae.

The hyphae of S. rolfsii are hyaline, thin walled and septate when young (Aycock, 1966). Higgins (1922) described two types of hyphae in the fungus: those composed of large cells with clamp connections at each septation, and the others consisting of small cells on which clamp connections were often absent.

2.2.3 Growth media.

Higgins (1927) observed that the growth of S. rolfsii on various vegetable decoctions was rapid and luxuriant with the formation of abundant sclerotia. In contrast, mycelial growth on several other media was poor and few or no sclerotia were formed.

2.2.4 pH of medium.

When grown in culture media in the presence of suitable carbohydrate sources, S. rolfsii causes a sharp decline in the pH of the medium. Final pH values of 2.0 to 4.0 have been recorded in culture media on which the fungus was growing (Johnson and Joham, 1954; Abeygunawardena and Wood, 1957). This rapid drop in the pH of the medium has been attributed to the production of oxalic acid by the fungus (Higgins, 1927; Avizohar-Hershenzon and Palti, 1962; Bateman and Beer, 1965).

2.2.5 Temperature requirement.

The minimum, optimum and maximum temperatures for mycelial growth reported for the fungus on agar medium are 8° to 10°C; 30° to 35°C, and 40°C respectively, with the maximum for normal hyphal growth at 37°C (Higgins, 1927; Abeygunawardena and Wood, 1957; Avizohar-Hershenzon and Palti, 1962). Similar temperature regimes also govern the formation, maturation and germination of sclerotia (Abeygunawardena and Wood, 1957).

2.2.6 Sclerotia formation.

Henis et al. (1973) showed that the formation of sclerotia in S. rolfsii was closely related to the pattern of hyphal branching at the colony margins. Other factors

such as nutrition (Wheeler and Sharan, 1965), chemical composition of medium (Wheeler, 1972) and physical factors such as light and temperature (Humpherson-Jones and Cooke, 1977) have also been reported to influence sclerotial formation in the fungus. In general, however, Wheeler and Waller (1965) concluded that lateral restriction of linear growth is an important condition for the formation of sclerotia in S. rolfsii.

When young, the sclerotia are white in colour and attached to the mycelium by a small stalk (Palo, 1933). As development progresses, they turn yellow, then gradually darken and at maturity, the sclerotia assume various shades of the brown colour becoming smooth and usually spherical in shape (Ezekiel and Taubenhaus, 1931; Braint, 1932; Aycock, 1966).

Johnson and Joham (1954) noted that the time of appearance of mature sclerotia gives the most uniform measure of maximum growth in S. rolfsii. Chet and Henis (1968) observed that the brown colour of mature sclerotia may be due to the presence of melanin-like pigments in the sclerotial walls. The size of mature sclerotia may be affected by the substrate used and/or the biotype of the organism under study (Aycock, 1966). Thus sclerotial size differs widely ranging from 0.1 to 10.0mm in diameter (Gloyer and Glasgow, 1924; Welch, 1924).

2.2.7 Variability.

The degree of variability which occurs within different strains of the fungus, led Wilson (1961) to describe S. rolfsii as "a well-known fungus with some well-kept secrets".

Various strains of the fungus have been distinguished on the basis of differences in size, shape, colour of sclerotia and tolerance to various pH levels (Higgins, 1927), as well as differences in the ability of the fungus to utilize certain carbohydrate sources (Abeygunawardena and Wood, 1957). Despite these differences which are usually small and inconsistent (West, 1961), S. rolfsii is fairly stable and uniform in culture media (Wolf, 1914; Higgins, 1927). However, Maduewesi (1976) observed some significant differences among different isolates of the fungus from various localities in eastern Nigeria.

The phenomenon of aversion which usually occurs between different strains of the fungus, but not between cultures of the same isolate, has been considered to be the only true evidence of variability within the species; S. rolfsii in particular (Epps et al., 1951) and the basidiomycetes in general (Dutt et al., 1974).

2.2.8 Ecology of *Sclerotium rolfsii*

The fungus is a soil-borne pathogen which survives in soil from one season to the other by the formation of sclerotia (Boyle, 1961; Chet *et al*., 1967; Addison and Chona, 1971). Boyle (1961) distinguished the mycelial or pathogenic phase of *S. rolfsii* from the sclerotial phase. He observed that the mycelial phase was governed, primarily, by physical conditions of the environment such as relative humidity and temperature, while the sclerotial phase depended on the amount and source of energy available, as well as on the character of the other micro-organisms present in the environment.

In nature, the growth of the fungus is almost always prominent at or near the soil surface, indicating that *S. rolfsii* has higher demands for oxygen than most other soil-borne micro-organisms (Watkins, 1961).

2.2.9 Survival of sclerotia of *Sclerotium rolfsii* in soil.

The survival of *S. rolfsii* in soil in the absence of host plant or organic residue is attributed to the sclerotia which act as organs of perennation. Sclerotia are known to survive in soil from a few weeks (Epps *et al*., 1971) to 10 years (Gould and Russell, 1965).

The ability of sclerotia of *S. rolfsii* to survive adverse conditions has been attributed to a variety of

factors. Chet et al. (1967) observed that the resistant melanins and melanin-like pigments contained in the sclerotial walls play an important role in the resistance of sclerotia to chemical and biological degradation. These workers also noted that the outer covering (rind) of mature sclerotia serve as a mechanical barrier preventing the uptake of nutrients from the medium. Chet (1969) reported that punctured sclerotia germinated more rapidly than unpunctured ones with the rind intact. These features may explain the ability of sclerotia of this fungus to pass through the digestive track of certain animals with little loss in viability as demonstrated by Leach and Mead (1936), and Akueshi and Oso (1976).

Physical and environmental factors such as relative humidity (Epps et al., 1951), temperature, soil moisture level, and oxygen concentration (Abeygunawardena and Wood, 1957) have also been found to influence the survival of both sclerotia and mycelium of S. rolfsii. Using field soil in growth chambers, Beute and Rodriguez-Kabana (1981) observed that high soil moisture levels favoured the survival of sclerotia at temperatures below 20°C than at or above 20°C. They also noted that the mycelium of S. rolfsii lost its viability rapidly in the presence of high soil moisture at all temperatures tested.

Novogrudsky (1948) observed that the capacity to produce antibiotics may protect some soil fungi from bacterial lysis. He also recorded differential resistance of fungal spores or sclerotia to toxin- and enzyme-induced lysis. These observations appear to explain the conclusions reached by Morton and Stroube (1955), and Cooper (1961) that S. rolfsii was relatively resistant to antagonism or parasitism by most soil micro-organisms.

2.2.10 Population and viability of sclerotia in soil.

Determination of the numbers and the viability (in the soil) of propagules of soil-borne plant pathogenic fungi is important for predictions on the severity of the diseases caused by these fungi in commercial fields. Several workers have employed different methods to achieve this purpose.

Maloy and Alexander (1958) developed the "most probable number" technique which gave good agreement between the populations of Fusarium solani (Burk.) Snyd. and Hans. and Thielaviopsis basicola (Berk. and Br.) Ferr. in the soil and diseases caused by these pathogens in the field In this method, suitable test plants are inoculated with equal portions of a dilution series of soil sample containing the inoculum. The number of plants showing infection at the different dilutions is then employed to obtain the most probable number of organisms in the original inoculum by using a table of "Most Probable Numbers".

The appearance of sclerotia as distinct morphological entities in the life cycle of certain fungi provides a unit of the pathogen which can be separated from soil, counted and its viability determined. Leach and Davey (1938) utilizing this advantage, recovered some viable sclerotia of S. rolfsii by washing soil samples through a series of sieves. Their results indicated a relatively high correlation between density of viable sclerotia in soil and percentage infection of sugar beets (Beta vulgaris L.) by S. rolfsii in the same area.

Other workers tested selective plant baits for the estimation of population density and viability of sclerotia of S. rolfsii in field soils. The results also showed good correlation between inoculum density of the pathogens and percentage colonization of such baits as carrot discs and sugar beet segments (Avizohar-Hershenzon and Shacked, 1968) as well as tissue blocks cut from the petiole of cocoyam leaf (Ikediugwu and Osude, 1977).

Rodriguez-Kabana et al. (1974) devised a "rapid flotation-sieving" technique for determining the populations of sclerotia of S. rolfsii in the soil. The method involved the flotation of sclerotia in a black strap molasses solution (specific gravity 1.073) at 25°C containing a flocculating agent or in a 0.75M sucrose solution of similar specific gravity. Recently, Rodriguez-Kabana et al. (1980) developed a relatively efficient (75%)

but less laborious method of estimating sclerotial numbers and viability in soil by the addition of methanol which stimulates dormant sclerotia in the soil sample to germinate after incubation.

2.2.11 *Dispersal of Sclerotium rolfsii.*

Sclerotia are the principal means of dispersal of S. rolfsii. Dissemination of the sclerotia may be by wind, water, or by contaminated soil adhering to farm implements, feet of animals and labourers' boots (Aycock, 1966). Dispersal of sclerotia can also occur through the faeces of certain animals previously fed on sclerotia alone (Akueshi and Oso, 1976) or on materials infested by the fungus (Mead and Davey, 1936). It has been demonstrated in various plants that the mycelium and sclerotia of S. rolfsii are borne on seeds (Emechebe and McDonald, 1979), on bulbs (Gould, 1954) and on tubers (Aycock, 1966). In such cases, the pathogen can be introduced into new areas by contaminated plant organs. It has, however, been suggested that the basidiospores may also play a role in the spread of the pathogen, although the ability of these propagules to infect host plants has not yet been established (Punja et al., 1982).

2.2.12 Geographical distribution of Sclerotium rolfsii.

Sclerotium rolfsii is world-wide in distribution, but particularly wide spread in the moist tropics and warm temperature areas (West, 1961; Mordue, 1974; Singh and Allen, 1979). Temperature relations, soil pH and soil moisture levels are regarded as critical factors in determining the geographical range of the pathogen. (Higgins, 1927; Epps et al., 1951).

The fungus occurs in most parts of Africa, southern Asia, South America, Australasia, central Europe, North America - particularly in the southern United States and in the West Indies (West, 1961; Mordue, 1974). In Africa, the pathogen has become a major problem of economically important crops especially in Nigeria (Quinn, 1971), Ethiopia (Mengistu, 1978), Egypt (Abdel-Hak et al., 1973), Tanzania (Clinton, 1957) and Sierra Leone (Deighton, 1955).

2.3 Infection of host plants by Sclerotium rolfsii.

2.3.1 Mode of infection.

The mode of infection of host plants by S. rolfsii is peculiar and fairly similar, irrespective of the plant being attacked. The plants are usually attacked at the base of the stems, near the soil surface, and the fungus gradually girdles the infected stem.

Higgins (1927) studied the parasitism of the fungus on several plants, and observed that the pathogen formed a mat over the infected portions of a host plant, and clung to the host epidermis by means of hold fasts. He also noted that the underlying cells were killed before the fungous hyphae entered the host tissue. However, S. rolfsii has also been reported as a leaf spot pathogen (Erinle, 1975), and a fruit pathogen (Quinn, 1971).

2.3.2 Pathogenesis of Sclerotium rolfsii.

The role of exogenous secretions in the pathogenesis of S. rolfsii has been stressed by several workers. Higgins (1927) observed that culture filtrates of the pathogen killed host plants and caused injuries similar to those caused by aqueous solution of oxalic acid or by the fungus itself. He also found considerable amounts of oxalic acid in host cells killed by S. rolfsii. He therefore attributed the death of host cells in advance of the penetrating fungal hyphae to the production of oxalic acid by the pathogen. Kritzman et al. (1977) reported reduced pathogenicity of the fungus on lima bean seedlings (Phaseolus limensis Macf.) when oxalic acid production by the pathogen was inhibited.

Milthrope (1941) demonstrated the presence of certain enzymes in the culture filtrates of S. rolfsii, and the importance of these enzymes in the pathogenesis of the fungus has been recognised (Johnson and Joham, 1954; Barnett, 1974). Thus Bateman and Beer (1965) suggested that oxalic acid, pectic enzymes and cellulase act synergistically in the destruction of host tissue by the pathogen.

2.3.3 Symptoms of the basal stem rot disease.

The first indication that a plant has been attacked by S. rolfsii is a wilting of the leaves in a manner very similar to fusarium and bacterial wilts (Weber and Ramsey, 1927; Braint, 1932). Young plants wilt suddenly and are killed, but older plants turn yellow and wilt before they die (Weimer, 1944; Averre and Jenkins, 1976; Wheeler, 1976).

The mycelium of the fungus often appears as a white feathery growth at the stem collar and gradually girdles the bases of dying plants. With the mycelium, small sclerotia are formed. The pattern of mycelial growth and the presence of sclerotia serve to distinguish the disease wherever it occurs (Weber and Ramsey, 1927; Ezekiel and Taubenhaus, 1931; Singh and Allen, 1979).

Although host plants are attacked at all stages of their growth, Weber and Ramsey (1927) observed that young tomato seedlings seldom showed disease symptoms in the field.

2.3.4 Factors affecting development of the basal stem rot disease.

The physical and environmental factors which affect mycelial growth, sclerotial formation and germination also influence infection and disease development in host plants. These factor include:

2.3.4.1 Soil moisture level.

In parts of the world where definite dry and wet seasons occur regularly, the basal stem rot disease is more prevalent in the wet season (Aycock, 1966). At this time, both soil moisture content and relative humidity are high in such areas. Severe outbreaks of the disease are normally associated with unusually wet season (Rosen and Shaw, 1929; Shay, 1953).

2.3.4.2 Temperature.

Atmospheric and soil temperatures of 20° to 35°C have been found to favour the disease on several crops (Epps et al., 1951; Onkarayya and Rao, 1970; Bell, 1974).

2.3.4.3 Shading.

Heavy shading and crowding of plants, which tend to increase the moisture content at or near the soil surface, favour disease incidence (Avizohar-Hershenzon and Palti, 1962; Aycock, 1966).

2.3.4.4 Inoculum potential.

Garrett (1970) and several other workers have emphasized the importance of adequate inoculum potential in the pathogenicity of soil-inhabiting fungi. Boyle (1961) pointed out that a supply of organic food base normally enabled the sclerotia of S. rolfsii to germinate and build up adequate inoculum potential necessary to parasitize a susceptible plant. However, it has been shown (Punja and Grogan, 1981b), that sclerotia germinating eruptively (Punja and Grogan, 1981a), were able to infect bean (Phaeseolus vulgaris L.) and sugar beet (Beta vulgaris L.) plants without an exogenous food base. Inoculum levels of 5 to 20% (W/W) were regarded as maximum for seedling infection of groundnut by S. rolfsii (Onkarayya and Rao, 1970).

2.3.4.5 Soil texture and pH.

Soil texture may also affect the ability of S. rolfsii to incite disease. Dubey (1958) reported a higher disease incidence in light sandy soil than in heavy clay soil.

He concluded that the lighter soil provided adequate soil aeration for this pathogen which appears to have a high demand for oxygen. Flados (1958) noted limited linear growth of S. rolfsii through heavy clay soils and attributed this to factors such as high moisture-holding capacity, poor aeration and small pore spaces in clay soil.

There is some doubt about the role of soil pH on the development of the basal stem rot disease. Higgins (1927) reported that the toxicity of his culture filtrate on tomato seedlings in pots, increased with increasing pH values of the filtrate. But Dubey (1958) observed that the occurrence of the disease in groundnut fields was independent of pH values.

2.3.4.6 Pre-Infection injury on host plants.

Mechanical injury on host plants has been reported to facilitate the entry of the pathogen into host tissues (Taubenhaus, 1919; Bell, 1974). Nematodes, which are well known wound agents, may also play a role in the pathogenicity of S. rolfsii. Reddy et al., (1972) pointed out that the root-knot nematode, Meloidogyne javanica (Treub) Chitwood, may be involved in a synergistic relationship with S. rolfsii in causing a disease complex in tobacco (Nicotiana tabacum L.).

Similar relationships between nematodes and various soil-borne pathogens had earlier been reported (Powell, 1963; Porter and Powell, 1967).

2.3.4.7 Soil micro-organisms.

Morton and Stroube (1955) have shown that micro-organisms which were antagonistic to S. rolfsii on agar plates also significantly reduced disease severity on soybean seedlings (Glycine max (L.) Merr.) in the greenhouse.

2.3.5. Host range of Sclerotium rolfsii.

Sclerotium rolfsii is one of the most destructive soil-inhabiting fungi because of its ability to attack a wide variety of plants, including those of economic values in many parts of the world (Higgins, 1927; West, 1961; Aycock, 1966).

Studies on the host range of the pathogen were first carried out by Rolfs who became aware of its potentially wide host range (Epps et al., 1951). Although primarily a pathogen of Angiosperms, S. rolfsii is known to attack several species of Bryophytes, Pteridophytes and Gymnosperms (Aycock, 1966).

Certain monocotyledonous plants and such dicotyledonous plants as cotton (Gossypium spp.), groundnut (Arachis hypogea L.) and sweet potato (Ipomoea batatas (L.) Lam.), which were earlier regarded as resistant to infection by the pathogen (Epps et al., 1951), are now known to be readily attacked by the fungus (Bailey, 1966; Abdel-Hak et al.,1973; Giha, 1977).

No comprehensive host list has been drawn up for the pathogen in Nigeria. Bailey (1966) listed 28 plant species attacked by the fungus in this country. Maduewesi (1975) reported a much wider host list for S. rolfsii in Nigeria. This list consisted of 116 susceptible plant species from 38 families and nine resistant species. Reports (Ebenebe et al., 1977; Giha, 1977; Emechebe, 1980) from the northern parts of the country, also indicate a widening host range for S. rolfsii in Nigeria.

2.3.6. Host resistance to Sclerotium rolfsii.

No variety of the cultivated tomato is known to be resistant to infection by S. rolfsii (Weber and Ramsey, 1927; Mohr and Watkins, 1959). But strain T-514 of Lycopersicon pimpinellifolium is reported to be resistant to attack by the pathogen (Mohr and Watkins, 1959).

Varietal resistance to S. rolfsii has however, been demonstrated in several other crops (Aycock, 1961; Garren, 1964; Mukhopadhyay, 1971). This has been attributed in some plants to the presence of thick-walled cortical cells (Higgins, 1927; Epps, et al., 1951).

2.3.7 Pattern of disease distribution in nature.

The distribution of the basal stem rot disease in nature is usually sporadic or discontinuous, with small numbers of infected plants scattered throughout an otherwise healthy stand (Higgins, 1927; Weimer, 1944; Shay, 1953). However, Averre and Jenkins (1976) reported a fairly uniform infection of tomato plants in the field, resulting in almost total destruction of the crop. Epps et al. (1951) suggested that the erratic disease occurrence in the field may be due to uneven distribution of the population of S. rolfsii in natural soil.

2.3.8. Sclerotium rolfsii as a general rot organism.

In addition to attacking plants in the field or greenhouse, S. rolfsii is a serious rot-causing agent. It has been implicated in the storage and market rots of several important food commodities in Nigeria.

The pathogen has been found to be responsible for the loss of over 20% of marketable tomato fruits at Ile-Ife (Onesirosan and Fatunla, 1976). The soft rot of yam tubers (Dioscorea spp.), cocoyam corms (Xanthosoma spp.) (Ogundana, 1970; 1976) and cassava tubers (Manihot esculentus Crantz) (Nwankiti and Arene, 1978) have also been attributed to S. rolfsii.

2.4 Control of the basal stem rot disease

Three approches have been followed in the difficult task of controlling the basal stem rot disease or its causal organism. These are:

A - Use of fungicides;

B - Use of biological control agents which are antagonistic to S. rolfsii,

C - Use of cultural methods during cultivation.

2.4.1 Chemical control.

2.4.1.1 Use of fungicides:

Since Rolfs tried to reduce crop losses due to this pathogen by the use of copper fungicides (Rolfs, 1893; 1913), several other attempts have been made to control the disease by the use of chemicals. Harrison (1961) suggested that the chemical control of the basal stem rot disease under field conditions required a

fungicide which is not adversely affected by extreme fluctuations in climatic and environmental conditions. He further stated that such a fungicide should be active and stable in the upper 1 to 5cm of soil. Such a fungicide was discovered when pentachloronitrobenzene (PCNB or quintozene), a volatile compound, was found to possess fungicidal activity against S. rolfsii (Harrison, 1961). With this discovery, the first real progress was made in the practical chemical control of the disease under field conditions (Aycock, 1966). There are reports of the successful use of PCNB for the commercial control of the basal stem rot disease in such crops as groundnut (A. hypogea) (Harrison, 1958), bulbous iris (Iris spp.) (Gould and Russell, 1965; Hass, 1976); tomato (Averre and Jenkins, 1976), and bentgrass (Agrostis palustris Huds.) golf greens (Punja et al., 1982a; Punja et al., 1982b). Other investigators have found the fungicide ineffective in the field on groundnut (Diomande and Beute, 1977) and apple trees (Malus domestica Borkh.) (Brown and Hendrix, 1980). Young (1960) reported some yield losses and stunted growth in tomato plants treated with PCNB. But Thomson (1973) considered the fungicide non-phytotoxic at normal rates of application.

Several other fungicides have also been reported to be effective against the disease on various crops in field plots. These fungicides include: potassium azide on groundnut (Rodriguez-Kabana and Backman, 1973), carboxin and triphenyl-tin-hydroxide (TPTH) on groundnut (Diomande and Beute, 1977) and captafol on apple (Brown and Hendrix, 1980). In greenhouse tests, Addison and Chona (1971) observed that mercuric chloride completely controlled the disease on young tomato seedlings in the greenhouse. Captan was also found to give effective control of the pre-emergence damping-off of sugar beet (Abegunawardena and Wood, 1957) and cowpea (Oladiran and Okusanya, 1980), caused by S. rolfsii but failed to control the post-emergence damping-off of cowpea seedlings (Oladiran and Okusanya, 1980).

In vitro, several fungicides have been shown to inhibit the growth of the pathogen although they were not necessarily effective in controlling the disease in greenhouse or field tests. These include; Ceresan, Agrosan GN, and captan (Mahmood et al., 1977), Mertect, triforine, Vitavax and Plantvax (Vyas and Joshi, 1977), Sicarol and Brestan which proved highly effective in killing the sclerotia of the fungus (Anilkumar et al., 1979) as well as carboxin (Diomande and Beute, 1977).

2.4.1.2. Volatile pesticides.

Soil fumigation with volatile pesticides have generally yielded good results in the effort to control S. rolfsii in farm lands. Thus chloro-bromopropene (CBP) (Young, 1954) and methyl bromide (MB) (Haas, 1976; Henis, 1978) have been effectively used to destroy the pathogen in field soils prior to planting or transplanting.

2.4.1.3 Herbicides.

Herbicides have also been employed in the control of S. rolfsii and other plant pathogenic fungi in field soils (Chappell and Miller, 1956). Such herbicides as EPTC (Rodriguez-Kabana et al., 1970) and fluometuron (Bozarth and Tweedy, 1971) have been shown to inhibit mycelial growth and sclerotia numbers of S. rolfsii in liquid cultures. Atrazine and paraquat have been reported to have similar effects on Rhizoctonia solani Kuhn (Rodriguez-Kabana et al., 1966).

2.4.1.4 Fungal resistance to fungicides.

Chemical control of plant diseases has been hampered by the ability of certain plant pathogenic fungi to develop resistance to fungitoxicants to which they were previously susceptible (Priest and Wood,

1961; Dekker, 1977). This phenomenon is wide spread in the aromatic hydrocarbon group of fungicides, particularly in the halogenated nitrobenzenes such as PCNB, tetrachloronitrobenzene (TCNB) and 2, 6-dichloro-4-nitroaniline (DCNA) (Georgopoulos and Zaracovitis, 1967). Resistance induced by this group of fungicides usually appears spontaneously in cultures of the fungus growing in high concentrations of the fungicide (Priest and Wood, 1961). The resistant fungal strain retained their resistance after long periods of growth in the absence of the fungicide. In contrast, resistance induced by other groups of fungicides is lost rapidly in the absence of the fungicide (Priest and Wood, 1961).

Certain strains of S. rolfsii and Fusarium solani (Mart.) Apple and Wr. were found to develop resistance to PCNB under laboratory conditions (Georgopoulos, 1965; Georgopoulos and Vomvoyanni, 1965). Georgopoulos and Zaracovitis (1967) also suggested that PCNB - resistance had developed in S. rolfsii in the field. Shatla and Sinclair (1963) reported highly significant differences in mycelial growth and pathogenicity of different isolates of Rhizoctonia solani in the presence of PCNB, a fungicide recommended for the control of cotton seedling diseases caused by the pathogen. Priest and Wood (1961) had also demonstrated resistance to PCNB and TCNB in Botrytis allii Munn.

2.4.2 Biological control.

The increasing demand for new fungicides to combat the menace of plant pathogenic fungi has introduced new and serious problems in the chemical control of plant diseases. Some of these problems include fungal resistance to fungicides (Dekker, 1982; Schwinn, 1982), high cost of application of fungicides and the toxicity of several fungicides to non-target crops and soil micro-organisms (Schwinn, 1982). These limitations of chemical control programmes seem to justify the search for alternative practical, more economical, more reliable and less hazadous methods of disease control. Considerable research has therefore been directed towards the biological control of plant diseases including the basal stem rot disease as described under the following headings:

2.4.2.1 Use of soil micro-organisms.

The direct and indirect use of soil microflora to influence the development and expression of different plant diseases had been widely reported. Ghaffar (1969a, 1969b) observed that Penicillium nigricans (Brain) Thom., was antagonistic to Sclerotium cepivorum Berk. on agar medium, and was also effective in controlling the white rot disease of onion (Allium cepa L.) incited by the pathogen. Several species of Aspergillus have been

found to retard the growth of S. rolfsii and other pathogenic fungi in culture and in soil (Aycock, 1966). Christias (1980) reported that sclerotial exudates of S. rolfsii were rich in protein and poor in carbohydrate content. He observed that in a nutrient-deficient soil environment, the nutrients released via sclerotial exudation may be available to sustain a wide variety of soil micro-organisms around the sclerotia. The activities of these sclerotial microflora may be detrimental to S. rolfsii, thus effecting natural biological control of the pathogen.

The genus Trichoderma appears to include several species capable of antagonizing plant pathogenic fungi. The importance of this group of antagonists in the biological control of plant diseases has been pointed out by many researchers. Weindling (1932, 1934) reported the antagonistic effect of T. viride Pers. ex S.F. Gray on S. rolfsii and R. solani in culture and greenhouse studies. Morton and Stroube (1955) tested over one thousand fungal isolates for antagonism towards S. rolfsii and observed that all the 35 isolates which inhibited S. rolfsii growth on agar plates and reduced disease severity in the greenhouse belonged to the genus Trichoderma.

Weindling (1932, 1934) attributed the effectiveness of T. viride in reducing the damping-off disease of citrus seedlings caused by R. solani to the production of a 'lethal principle' by T. viride. This 'lethal principle' was later isolated and named 'Gliotoxin' (Weindling and Emerson, 1936). The production of another antibiotic named viridin was also ascribed to T. viride (Brian and McGowan, 1945; Brian et al., 1946). However, Webster and Lomas (1964) re-examined the gliotoxin-and viridin-producing isolates and concluded that these organisms were not T. viride but rather isolates of Gliocladium virens.

More recent studies on the antagonistic action of T. viride against S. rolfsii in mixed cultures of both organisms in soil, indicate that T. viride produces proteolytic enzymes which degrade and stop the activity of saccharase enzymes produced by S. rolfsii (Rodriguez-Kabana and Curl, 1968). It was further observed that T. viride increased the pH of the medium to levels unsuitable for enzymatic activity by the pathogen (Rodriguez-Kabana, 1974). Elad et al. (1983) observed that antagonistic Trichoderma spp. were attached to hyphae of S. rolfsii by hyphal coils, hooks or appressoria and noted lysed sites and penetration holes by T. harzianum Rifai in hyphae of the plant pathogenic fungus.

The first successful field control of a soil-borne plant pathogen by means of biological agents introduced into soil was reported by Wells et al. (1972). They achieved disease control by delivering 4,200 kg/ha of organic matter, artificially inoculated with the antagonist, T. harzianum, into groundnut fields infested by S. rolfsii. Later, Backman and Rodriguez-Kabana (1975) employed a diatomaceous earth granule impregnated with 10% molasses solution for the growth and delivery of T. harzianum to infested groundnut fields. But Punja et al. (1982b) found that Trichoderma spp. which antagonized S. rolfsii in vitro failed to control the disease on bent grass.

2.4.2.2 Use of soil amendments.

The use of biological control agents for large scale field control of soil-borne plant pathogens can be achieved indirectly by employing various beneficial soil amendments. These amendments have the effect of increasing the population of soil micro-organisms antagonistic to the pathogen as well as of altering host plant metabolism or affecting the ability of the pathogen to grow and cause disease (Leach and Davey, 1942). Various nitrogenous amendments have been found to reduce the basal stem rot disease on sugar beets in

the field (Leach and Davey, 1942; Thakur and Mukhopadhyay, 1972). In pot experiments (Johnson (1953) and Henis and Chet (1968) observed a marked destruction of the sclerotia of S. rolfsii in the presence of different nitrogenous amendments. They also attributed the destruction of the sclerotia to an increase in the numbers and activity of micro-organisms antagonistic to S. rolfsii in the presence of these amendments.

Amendments for the control of the basal stem rot disease may be incorporated into soil in the form of organic matter such as crop residues (Huber et al., 1965; Mixon and Curl, 1967), green manures (Huber and Watson, 1970) or animal manures (King and Loomis, 1926; Fellows, 1929). Inorganic amendments may be incorporated in the form of relatively pure sources of carbon and nitrogen (Maurer and Baker, 1965; Green and Papavizas, 1968), which are the major nutrients of fungi. However, some workers have been unable to correlate nitrogen application with the control of the basal stem rot disease. In an unpublished paper cited by Avizohar-Hershenzon and Palti (1962), Lavee observed no beneficial effect of nitrogen application on the disease in Israel.

Mohr and Watkins (1959) reported good control of the basal stem rot disease on tomato by the application of calcium nitrate. They attributed the beneficial effect of this amendment to the role of calcium in periderm formation in young plants and concluded that the periderm barrier was the effective mechanism of resistance to the disease in tomato plants.

2.4.3 Control by cultural methods.

The obvious limitations in the chemical and biological control methods, had led to the search for yet cheaper and more practical measures for combating the menace of S. rolfsii on crops in the field. Thus different cultural methods have been adopted for various crops in the effort to control the basal stem rot disease. Jackson and Bell (1969) asserted that cultural practices were very successful in the control of the disease on groundnut. The following cultural practices will be examined:

2.4.3.1 Methods of cultivation.

Most of the methods of cultivation reported for the control of the basal stem rot disease appear to be concerned with the destruction of the sclerotia of the incitant, S. rolfsii. These methods also recognise the importance of organic matter in the ecology of the

pathogen and the fact that the fungus is active mainly at or near the soil surface (Garren, 1961). Several researchers working on groundnut, have recommended different cultural methods for the elimination of the disease on this crop. Some of these practices include:

(i) deep ploughing to remove organic matter from the soil surface and bury it below the crop's infection zone (Taubenhaus, 1919; Bottomley, 1940),

(ii) avoiding the piling up of soil around the bases of groundnut plants during cultivation (non dirting), thus preventing the formation of new organic debris by branches and weeds smothered with soil (Garren and Duke, 1957; Garren, 1959; 1961) and

(iii) effective control of foliar diseases to prevent leaf drop and subsequent accumulation of dead leaves at the bases of groundnut plants (Garren, 1959, 1961).

The tillage procedures which have been advocated by various workers were aimed primarily at the elimination of the sclerotia of S. rolfsii. Tisdale (1921) suggested early ploughing followed by thorough disking in order to promote the germination and exhaustion of sclerotia. Bottomley (1940) suggested stirring the soil immediately after harvesting groundnut to promote drying out of immature sclerotia. In Nigeria, Maduewesi (1975) suggested early ploughing of the fields and flooding with hot water to kill the sclerotia. Flooding of farm lands in India (Vasudeva, 1955) and the introduction of cover crops in Israel (Avizohar-Hershenzon and Palti, 1962) have been practiced as cultural control measures against the basal stem rot disease.

2.4.3.2 Crop rotation.

The use of crop rotation as a means of controlling the basal stem rot disease has met with little success. This is due, mainly, to the very wide host range of S. rolfsii (Garren, 1957). The ability of the fungus to survive for long periods in the soil as a saprophyte on plant debris or in the form of sclerotia in the absence of the host plant has also helped to render crop rotation almost ineffective in the control of this disease (McClintock, 1917; Garren, 1959). Considering

the sporadic nature of the disease in the field (Averre and Jenkins, 1976), occurring in some years and not in some others on the same plot, it would be difficult to give any credit to crop rotation as a control measure, except after several years of repeated trial. But enough data from such rotation studies do not seem to have been published (Garren, 1961).

However, there are reports of reduced incidences of the basal stem rot disease on groundnut after 1-to 4-year (Garren, 1961) and 1-to 7-year (Cooper, unpublished) rotations with less susceptible crops. Young (1954; 1960) achieved almost complete control of the disease on tomato in a 5-to 8-year rotation with grasses and rye (Secale cereale L.), but observed a severe infection when tomato followed cotton (Gossypium sp.) or corn (Zea mays L.) in a rotation. Report (Erinle, 1980) from Samaru suggests that the disease is often serious when tomato follows groundnut.

2.4.3.3 Mulching, staking and other cultural practices.

Quinn (1974, 1977) has shown that mulching and staking of tomato plants reduced the incidence of the basal stem rot disease on rain-fed crop in Samaru.

Other cultural methods adopted for the control of the disease include: non-tillage practices which have been reported to reduce losses in apple due to S. rolfsii in Israel (Avizohar-Hershenzon and Palti, 1962), solar heating, reported to control S. rolfsii and other soil-borne pathogens as well as weeds in field plots in Israel (Grinstain et al., 1979). Solar heating consisted of mulching infested groundnut plots with transparent polyethylene sheets during the hot season. Such treatment was found to reduce disease and increase total groundnut yield by about 52%, and has been recommended for use in crops grown in hot regions.

CHAPTER 3

3.0 DISEASE SURVEY IN THE FIELD

3.1 Materials and Methods

3.1.1 Area covered by the survey.

The survey for the basal stem rot disease in this study was carried out in parts of northern Nigeria between November 1976 and August 1981. The main centres of investigation were located in the Northern Guinea and Sudan Savanna Zones lying between 9° and 12°N. But two localities in the Southern Guinea Savanna Zone were also included (Fig. 1). These areas show marked seasonal differences in weather conditions during the year which consists of a wet season (May to October) and a dry season (November to April).

3.1.2 Sites visited.

At the start of this investigation, information was scanty on the geographical distribution of *S. rolfsii* in northern Nigeria. Visits to several farms of tomato and other crops in the various parts of the ecological zones under study were therefore conducted. A total of 16 localities (Table 3.1) were visited, and three farms were examined in each locality. Eleven of these localities were upland areas sampled during both the wet and dry seasons, while five sites were low lying (fadama) areas.

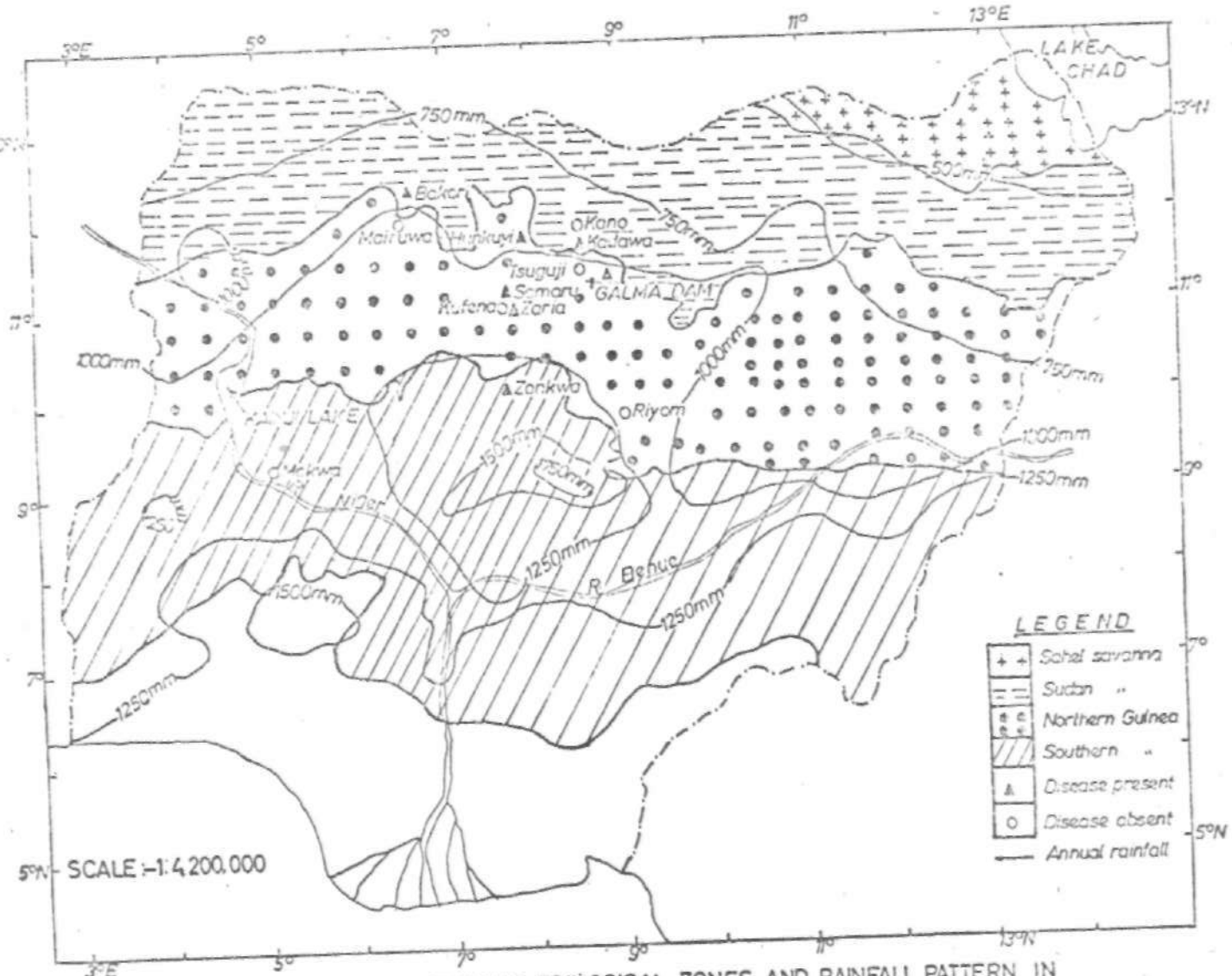


Fig. 1: MAP OF NIGERIA SHOWING ECOLOGICAL ZONES AND RAINFALL PATTERN IN NORTHERN NIGERIA AS WELL AS THE DISTRIBUTION OF BASAL STEM ROT DISEASE IN PARTS OF NORTHERN NIGERIA. (Source :-Soil survey unit, ABU Zaria, Nigeria)

TABLE 3.1

Localities visited during the survey for the basal stem rot disease caused by Sclerotium rolfsii in parts of northern Nigeria, during the period November 1976 to August 1981.

Ecological Zone	Localities visited	Dates visited	Season	Nature of site	Crops grown in the locality	Disease present/ absent
1. +N.G.S.	Samaru I, (Institute for Agric. Research farms)	18/11/76	dry	Upland	tomato and onion	absent
		26/2/77	dry	Upland	tomato	absent
		12/6/77	wet	Upland	tomato, groundnut, onion, maize	absent
		3/8/77	wet	Upland	sorghum, tomato, millet, onion, groundnut	present on tomato
		6/3/78	dry	Upland	onion, egg plant, tomato	present on tomato
		16/8/78	wet	Upland	groundnut, sorghum, tomato, onion, millet	present on tomato in field, onion in storage.
		28/8/79	wet	Upland	cowpea, groundnut, cotton, tomato, sorghum	present on tomato
		20/3/80	dry	Upland	tomato	absent
		7/7/80	wet	Upland	groundnut, cowpea, sorghum, tomato	present on tomato
		12/8/81	wet	Upland	egg plant, spinach, potato, tomato	present on tomato
2. N.G.S.	Samaru II, (Area near Ahmadu Bello University dam)	20/3/78	dry	Fadama	pepper, potato, tomato, cabbage	present on tomato
		10/4/79	dry	Fadama	maize, cabbage, groundnut, tomato	absent
3. N.G.S.	Tsuguji	18/11/76	dry	Fadama	potato, tomato, pepper, okra	absent
		15/3/78	dry	Fadama	cabbage, tomato, potato	absent
4. N.G.S.	Mairuwa dam, Funtua	12/9/79	dry	Fadama	millet, sorghum, maize	absent
5. N.G.S.	Galma I	21/8/78	wet	Upland	potato, pumpkin, squash gourd, tomato	absent
		5/7/79	wet	Upland	cabbage, maize, groundnut, potato, tomato	absent
6. N.G.S.	Galma II	10/4/78	dry	Fadama	tomato, cabbage, potato, onion, okra	present on cabbage.

Table 3.1 (cont'd)

Ecological Zone	Localities visited	Dates visited	Season	Nature of site	Crops grown in the locality	Disease present/ absent
7. N.G.S.	Hunkuyi I	22/8/78	wet	Upland	groundnut, tomato, onion, potato, yam	present on cabbage
		6/9/79	wet	Upland	onion, maize, tomato, cabbage, potato	present on tomato
		25/8/80	wet	Upland	carrot, cabbage, tomato, onion, pepper	present on tomato
8. N.G.S.	Hunkuyi II	17/3/79	dry	Fadama	carrot, tomato, cocoyam	absent
		14/3/80	dry	Fadama	tomato, cocoyam, groundnut, carrot	absent
9. N.G.S.	Zaria	21/9/79	wet	Upland	groundnut, tomato, onion, potato	present on tomato
		8/8/80	wet	Upland	squash gourd, pumpkin, tomato, carrot	present on pumpkin
10. N.G.S.	Kufena	13/9/79	wet	Upland	squash gourd, onion, sorghum, potato	absent
11. N.G.S.	Riyom	27/7/80	wet	Upland	tomato, potato, groundnut, maize	absent
12. S.G.S.	Mokwa	20/9/79	wet	Upland	sorghum, potato, groundnut, tomato	absent
13. +S.G.S.	Zonkwa	11/9/80	wet	Upland	pumpkin, potato, tomato, groundnut	present on potato
14. S.S.	Bakori	3/5/78	dry	Upland	cabbage, potato, tomato, okra	present on tomato
		20/8/78	wet	Upland	tomato, potato, cabbage, groundnut	present on tomato in field, potato in storage.
15. ##S.S.	Kadawa	25/9/79	wet	Upland	potato, onion, tomato, groundnut	absent
		17/3/80	dry	Upland	tomato, cabbage, onion, potato, pepper	present on tomato
16. S.S.	Kano	25/9/79	wet	Upland	onion, pumpkin, groundnut, tomato	absent.

\+ N.G.S. = Northern Guinea Savanna
\# S.G.S. = Southern Guinea Savanna
\## S.S. = Sudan Savanna

The fadama sites were usually close to rivers or streams and were surveyed only during the dry season as they were flooded during the wet season. In the dry season, these fadama areas are usually cultivated under irrigation by water pumps or by shadoof.

3.1.3 Disease evaluation.

In all fields visited, disease incidence and severity on the crop were visually assessed. A plant was considered to be diseased if the stem or one or more branches were completely or partially girdled by mycelium of S. rolfsii. Fruit rot due to the pathogen was also considered to be an attack on the plant (Sitterly, 1962).

Disease incidence on a particular crop in the field was estimated by counting the number of plants infected in a given area of the field selected at random, and expressing this figure as a percentage of the total number of plants sampled within this area. Disease severity on each crop was assessed by giving each diseased plant a numerical rating similar to that used by Adeniji et al. (1975). The numerical ratings employed in this investigation were:

0 = No visible S. rolfsii attack.

1 = >0% but < 25% of stem or branch circumference girdled, or fruit attacked.

2 = 26 to 75% of stem or branch circumference girdled, or entire fruit rotted.

3 = 76 to 100% of stem or branch circumference girdled, or branch wilted.

4 = Plant wilted.

5 = Entire plant dead.

The disease severity index (D.S.I.) of S. rolfsii attack for each crop was worked out as follows:

$$\text{D.S.I.} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of infected plants} \times 5} \times 100$$

5, being the highest numerical rating recorded (Ebenebe and Fehrmann, 1974).

3.1.4 Collection of plant samples from the field.

In an affected field, five to ten plants showing disease symptoms, and one to five plants showing no symptoms, were randomly identified. This was done by tagging two plants (one of which showed disease symptoms, while the other did not) within every 10sq metre of the field until the required number of each type of plant was obtained. The tagged plants were later dug up with as much of their roots as possible, placed in separate clean polyethylene bags and brought back to the laboratory for further investigation.

3.2 Results of disease survey in the field.

3.2.1 Crops attacked in the field and in storage by Sclerotium rolfsii.

During the period of the survey, the basal stem rot disease was recorded on five crops (Table 3.2). These were tomato (Lycopersicon esculentum Mill.); cabbage (Brassica oleraceae L.); and pumpkin (Curcubita moschata (Lam.) Poir). The pathogen was also found to attack onion (Allium cepa L.) bulbs and potato (Solanum tuberosum L.) tubers in storage in this survey. Tomato was by far the most susceptible crop to the disease in the areas covered during the survey. Throughout the period of the survey, the disease was found only on mature or maturing crops bearing fruits. No disease symptoms were noticed on seedling plants in the field.

3.2.2 Description of field symptoms of the basal stem rot disease on tomato plant.

The symptom descriptions presented here were based mainly on observations made in Samaru and Hunkuyi which were visited more often than other locations.

Two phases of the pathogen were observed on infected tomato plants in the field: the mycelial or early phase and the sclerotial or late phase.

TABLE 3.2

Incidence of the basal stem rot disease caused by Sclerotium rolfsii on some wet and dry season crops in parts of northern Nigeria during the period, 1976 to 1981.

Localities visited and year		DISEASE INCIDENCE (%)								
		Wet Season						Dry Season		
		Tomato	Potato	Groundnut	Pumpkin	Onion	Squash gourd*	Tomato	Potato	Cabbage
1. Samaru I,	1976	*NV	NV	NV	NV	NV	NV	0.0	NV	NV
	1977	2.0	0.0	0.0	NV	0.0	NV	0.0	NV	NV
	1978	22.0	0.0	0.0	NV	2.0	NV	5.0	NV	NV
	1979	60.0	NV	0.0	NV	0.0	NV	NV	NV	NV
	1980	3.0	NV	0.0	NV	NV	NV	0.0	NV	NV
	1981	5.0	0.0	NV	NV	NV	NV	NV	NV	NV
2. Samaru II,	1978	NV	NV	NV	NV	NV	NV	1.0	0.0	0.0
	1978	NV	NV	NV	NV	NV	NV	0.0	NV	0.0
3. Tsuguji	1976	NV	NV	NV	NV	NV	NV	0.0	0.0	NV
	1978	NV	NV	NV	NV	NV	NV	0.0	0.0	0.0
4. Bakori	1978	7.0	NV	NV	NV	NV	NV	50.0	2.0	0.0
5. Galma I,	1978	0.0	0.0	NV	0.0	NV	0.0	NV	NV	NV
6. Galma II,	1978	NV	NV	NV	NV	NV	NV	0.0	0.0	2.0
7. Hunkuyi I,	1978	10.0	0.0	0.0	NV	0.0	NV	NV	NV	NV
	1979	15.0	0.0	NV	NV	0.0	NV	NV	NV	NV
	1980	4.0	NV	NV	NV	0.0	NV	NV	NV	NV

NV = Not investigated

Table 3.2 (Cont'd)

Localities visited and year		DISEASE INCIDENCE (%)								
		Wet Season						Dry Season		
		Tomato	Potato	Groundnut	Pumpking	Onion	Squash gourd	Tomato	Potato	Cabbage
8. Hunkuyi II,	1979	0.0	NV	NV	NV	NV	NV	0.0	NV	NV
	1980	0.0	NV	0.0	NV	NV	NV	0.0	NV	NV
9. Zaria	1979	2.0	0.0	0.0	NV	0.0	NV	NV	NV	NV
	1980	0.0	NV	NV	0.5	NV	0.0	NV	NV	NV
10. Kufena	1979	NV	0.0	NV	NV	0.0	0.0	NV	NV	NV
11. Kadawa	1979	0.0	0.0	0.0	NV	0.0	NV	NV	NV	N V
	1980	NV	NV	NV	NV	NV	NV	5.0	0.0	0.0
12. Kano	1979	0.0	NV	0.0	0.0	0.0	NV	NV	NV	NV
13. Mokwa	1979	0.0	0.0	0.0	NV	NV	NV	NV	NV	NV
14. Zonkwa	1980	0.0	3.0	0.0	0.0	NV	NV	NV	NV	NV
15. Riyom	1980	0.0	0.0	0.0	NV	NV	NV	NV	NV	NV

The mycelial phase of the pathogen was the first to appear in the field and was usually short-lived. White feathery mycelium of S. rolfsii was found growing rapidly on and usually encircling the bases of infected stems near the soil line, or affected branches and fruits in contact with soil (Plate I). Girdling by the pathogen led to the early death and eventual decay of the outer tissues of infected plant parts.

The profuse mycelial growth of S. rolfsii usually associated with the mycelial phase of the disease (Plate II), was most evident and persistent in plots where the tomato plants were crowded together to form a low 'thick bush'. Under such humid environment with high percentage vegetative cover and consequently high relative humidity, the pathogen spread rapidly from one plant or branch to the other. Initially few plants were killed, and few sclerotia formed. This pattern of disease development was more noticeable when bright sunshine accompanied by high temperatures (25^{o} - 35^{o}C) occurred one or two days after rainfall.

The life-span of the mycelial phase of the pathogen depended on the prevalence of high relative humidity (80 - 100%) and high atmospheric temperatures (25^{o} - 35^{o}C). If these factors persisted, this phase of the pathogen lasted longer. A dry spell



Plate I. Tomato plant naturally infected by the basal stem rot disease during the wet season in Samaru, showing withered leaves and branches and the white mycelium of Sclerotium rolfsii attacking stem, fruits and branches in contact with infested soil.



Plate II. Tight webs of the mycelium of Sclerotium rolfsii around naturally infected branch of tomato plant during the early or mycelial phase of the basal stem rot disease in Samaru.

of two or more days during the wet season usually marked the end of the mycelial phase of the pathogen and the onset of the sclerotial phase. Irregular or insufficient irrigation during the dry season also had the same effect. Just before the end of the mycelial phase, the mycelium of S. rolfsii became less noticeable on the surface of host plants or on soil. Numerous sclerotia appeared and the fungal mycelium began to disintegrate.

The sclerotial phase of the pathogen followed closely upon the mycelial phase. The sclerotial phase was marked by the formation of numerous sclerotia (Plate III) by the fungus. Young sclerotia were white in colour but later changed to a predominantly brownish colour. The sclerotia usually lay singly on the soil surface or on infected plant parts, but some times a few sclerotia clumped together into a small mass. No sclerotia were found on the living tissues of infected plants. These sclerotia served as important diagonistic features of the disease in the field.

The sclerotial phase of the pathogen was dominant and readily observed in plots with scanty crop stand or during long periods when low relative humidity (45 - 60%) and high atmospheric temperatures (25° - 35° C)



Plate III. Numerous brownish sclerotia of Sclerotium rolfsii formed on naturally infected branch of tomato plant during the late or sclerotial phase of the basal stem rot disease in Samaru.



Plate III. Numerous brownish sclerotia of Sclerotium rolfsii formed on naturally infected branch of tomato plant during the late or sclerotial phase of the basal stem rot disease in Samaru.

prevailed. The end of this phase of the pathogen came when most of the sclerotia were detached from the host plant, probably shaken off by wind, rain or animals. At this stage, particularly in maturing plants, the entire diseased plant or parts of it had withered and died. In mature and fruiting plants, however, some infected stands remained green with long brown or dark-brown nectrotic lesions in the affected parts, as the only remaining signs of infection by S. rolfsii.

3.2.3 Stem and branch symptoms.

Tight webs of the mycelium of S. rolfsii were often found at the stem and branches of infected tomato plants during the early stages of infection (Plate II). In one instance, the mycelium of the pathogen was observed on branches of tomato plants at a height of 18 to 40 cm above soil level.

Fungal mycelium was also found to creep on the soil surface from the bases of nearby diseased plants or along lower branches (Plate I) in contact with infested soil. In this way, the fungus was observed to spread to a distance of 10 to 13 cm from the bases of the originally infected plants in a field in Bakori (Kaduna State).

The outer cortical tissues of stem bases were usually attacked first, decayed and were readily peeled off, to expose the woody tissues (Plate IV). When split open, the xylem tissues of diseased stem were brown in colour but no mycelium or sclerotia of S. rolfsii were found within them. In some cases, after the tomato stem was girdled by the pathogen, the plant survived by producing adventitious roots above the damaged zone.

3.2.4 Root symptoms.

Throughout this investigation, infection of tomato roots by S. rolfsii was observed only once in an irrigated field in Bakori. Mycelial webs similar to those on diseased stems and branches were found on the roots of plants whose stems and branches were already dead. As on the stems, the presence of these mycelial webs appeared to have led to the decay of the outer tissue (piliferous layer) of the roots. In the Bakori plot, infected roots and actively growing mycelium of the pathogen were recovered at a depth of 20 to 25cm.

3.2.5 Fruit symptoms.

Sclerotium rolfsii attacked tomato fruits at all stages of development causing severe rot. Infection started at any point on the fruit which came into contact with



Plate IV. (A) Healthy tomato plant.

(B) Tomato plant, artificially inoculated with Sclerotium rolfsii showing withered leaves and branches, and clearing of the stem base (arrowed) due to the basal stem rot disease.

infested material. Once the pathogen was established, it spread rapidly on the outer surface of the fruit, initially in a star-shaped form, similar to the pattern of spread of the fungus in artificially inoculated tomato fruits (Plate V). Later, the infection developed into a watery lesion which gradually increased in size, until all or most of the fruit was rotted and turned into a mass of sclerotia (Plate VI).

3.2.6 Leaf symptoms.

No cases of the infection of tomato leaves by S. rolfsii were found in the field during this survey.

3.2.7 Description of field symptoms of the basal stem rot disease on other crops.

Symptoms of the basal stem rot disease on other susceptible crops were largely similar to those on tomato. The pathogen attacked onion bulbs and potato tubers, displaying webs of white ropy mycelium on the surfaces of these plant materials. In both organs, the affected parts were initially dry, but gradually softened. These plant organs appeared to be less readily invaded by S. rolfsii than tomato fruits. The mycelium of the pathogen was observed on the leaf-blades of 'bahama' grass (Cynodon dactylon L.) which, however, did not



Plate V. Tomato fruit, artificially inoculated with Sclerotium rolfsii, showing the star-shaped form of the pathogen usually observed during the early stages of fruit infection.

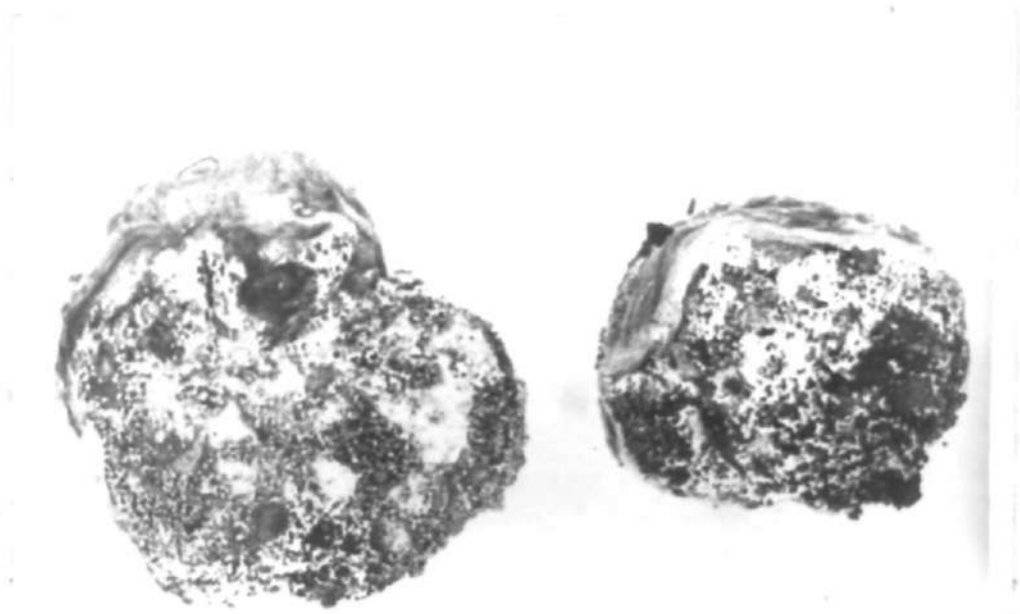


Plate VI. Tomato fruit, naturally infected by *Sclerotium rolfsii* in an advanced stage of decay, showing entire fruit consisting of a mass of sclerotia.

show any disease symptoms. In a dry season survey in Zaria, infected pumpkin fruits were found in which the pathogen with its numerous sclerotia was lodged in a depression on the fruits. A small water-soaked lesion, 2 to 3mm wide, was first observed around the depressed area; the lesion then spread rapidly under moist conditions (Plate VII).

3.2.8 Disease incidence and severity.

The basal stem rot disease was observed in various fields throughout the year, attacking rainfed crops such as tomato and pumpkin during the wet season and crops grown under irrigation such as cabbage during the dry season (Table 3.2).

The occurrence of the disease in nature was erratic. Thus, tomato plots in which S. rolfsii caused severe damage in one year or growing season, may be free from the disease during the next few years or seasons. This pattern of disease occurrence was observed even when such plots were cropped to tomato or other susceptible crops in the successive years or seasons. Two patterns of disease distribution were observed in infected tomato plots. In some cases, few diseased plants were scattered and widely separated by apparently healthy

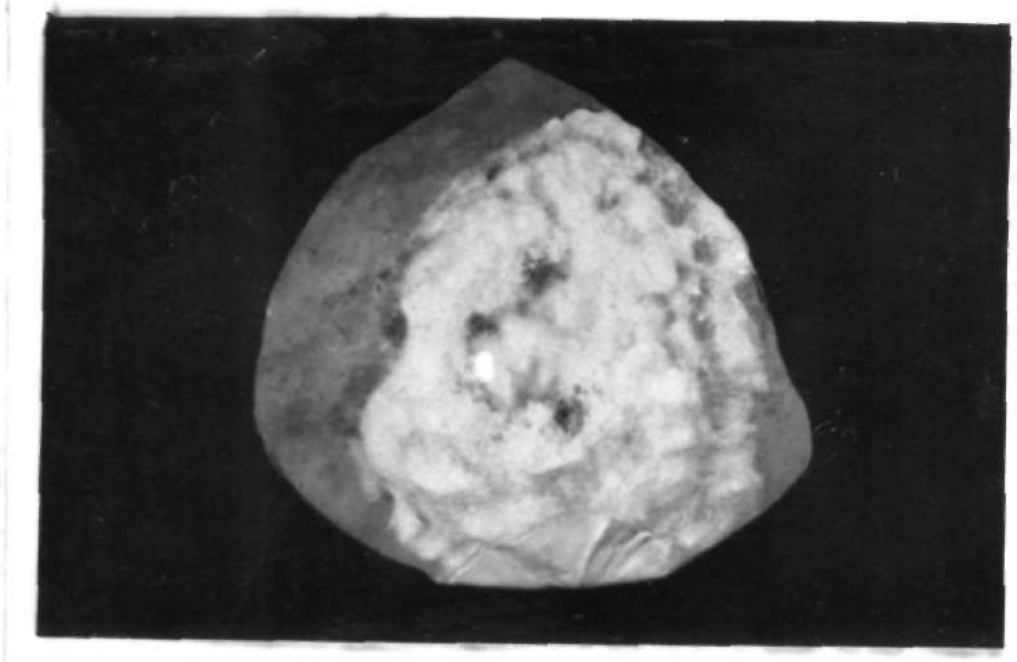


Plate VII. A section of pumpkin fruit naturally infected by Sclerotium rolfsii showing white, dense, mycelium of the fungus after 3 days of incubation in a moist chamber.

plants of the same or different species. In other cases, the disease occurred in rows sometimes with up to 10 consecutive plants in a row showing disease symptoms.

During the 1978 wet season, 50 to 60% of total fruit production was destroyed by S. rolfsii in a tomato farm in Hunkuyi. Earlier, in the dry season of the same year, similar losses were recorded on the tomato crop grown under irrigation in Bakori (Table 3.2). During this survey, the lowest and highest disease incidences on tomato stems were recorded in Samaru during the wet seasons of 1977 and 1979 respectively (Table 3.3). Table 3.3 also indicates that the lowest and highest mean annual rainfall figures for Samaru in 5 years (1977 - 1981) were recorded during the same periods.

The basal stem rot disease was equally severe on crops during the wet and dry seasons. The highest disease severity ratings of 4 and 5 were recorded on tomato in places such as Samaru I (Institute for Agricultural Research farm), Bakori and Kadawa during both the wet and dry seasons, and on cabbage in Galma II during the dry season (Table 3.2).

TABLE 3.3

Incidence of the basal stem rot disease on tomato in 5 fields at the Institute for Agricultural Research Farm, Samaru, from May 1977 to August 1981, in relation to rainfall in Samaru over the same period.

Mean atmospheric temperature during this period was: 24.9°C.

Field	Year	Annual rainfall (mm)	Disease incidence (%)
BFD	1977	745.5	2.0
BFA	1978	1148.9	22.0
X2	1979	1193.7	60.0
U4	1980	847.4	3.0
W6	1981	1019.1	5.0

Figures for the six rainy months May - October.

**Rainfall figures were supplied by the Meterological unit of the Institute for Agricultural Research, Samaru - Zaria.

CHAPTER 4

4.0 LABORATORY STUDY OF ASPECTS OF THE BIOLOGY OF SCLEROTIUM ROLFSII.

4.1 Materials and Methods

4.1.1 Microscopic examination of diseased plant materials from the field.

Diseased plant materials brought back from the field were first examined in the laboratory using X 10 objective of the dissecting microscope. Some of these plant materials were later incubated in clean moist polyethylene bags for 3 days at room temperatures (25^{o} to $28^{o}C$). This was done in order to determine the presence of Sclerotium rolfsii and other fungi present on tomato plants affected by the basal stem rot disease.

4.1.2 Isolation of fungi from diseased and non-diseased plant materials from the field.

The roots, stems and fruits of tomato and other plants showing disease symptoms in the field and those without symptoms brought back from the field, were shaken free of soil and thoroughly washed under a jet of tap water to remove adhering soil and debris. After washing, the various plant parts were cut into segments with a sharp scapel. For the diseased materials,

the segments were taken from the advancing margin of infected organs. In the case of plants without symptoms, sections were cut from apparently healthy portions. Cut plant segments were then surface sterlized by immersing them in 0.1% mercuric chloride solution for 30 seconds, and washing in three changes of sterile distilled water in McCartney bottles. Diseased and non-diseased segments were sterilized in separate bottles. After surface sterilization, all plant segments were dried (separately) between sheets of sterile filter paper (sterilised as described on page 65) and aseptically placed (3 pieces per plate) on one day-old potato dextrose agar (PDA) medium in 9.00cm Petri dishes. The Petri dishes were then incubated for three days at 30 $\pm$ 1°C. At the end of the incubation period, fungal colonies growing on the agar medium or on plant segments were aseptically transferred (using flamed and cooled inoculating needle) to fresh PDA, until pure cultures of all isolates were obtained. Plant materials not immediately needed for isolations were kept at 25° to 28°C till required (one to two weeks later).

In the case of fruit infection, the entire fruit was swabbed with 75% ethanol and the skin around the rotted area pulled back with sterile forceps. Mesocarp

from rotted and marginal areas of the rot was then transferred into PDA plates (Onesirosan and Fatunla, 1976). Mesocarp from healthy fruits was similarly plated. Isolations were also made from sclerotia collected from roots, stems and fruits of naturally infected tomato plants. Such sclerotia were washed in three changes of sterile distilled water, dried between sheets of sterile filter paper before plating (10 per plate) on PDA dishes. All dishes were incubated at 30 $\pm$ 1°C for 3 days. Fungi developing from both plant material and sclerotia were transferred into fresh PDA until pure cultures of all isolates were obtained.

Stock cultures were stored on PDA slants in McCartney bottles at 15° to 18°C until needed. They were renewed every two or three months by transferring pieces of mycelium or whole sclerotia to fresh PDA slants. Inoculum used in experiments in this study were always taken from 3 day-old cultures made from the original isolate.

4.1.3 Identification of fungal isolates.

The identification of all fungal cultures isolated from field materials was done by reference to Bessey (1950), Gilman (1957), Barnett (1960) and Funder (1961); alternatively, the cultures were sent to the Commonwealth Mycological Institute (C.M.I.), Kew, for identification.

A total of 14 isolates of S. rolfsii, obtained from various hosts in different parts of northern Nigeria, were identified and used in this study (Table 4.1).

4.1.4 Sterilization of media and glasswares.

Before use, the various agar media employed in this study, were dispensed in 250ml Erlenmeyer flasks plugged with cotton wool which was then wrapped in aluminium foil. These flasks were autoclaved for 15 minutes at 1 kg/cm^2 pressure. Filter papers were wrapped in aluminium foil and then sterilized by autoclaving. Glasswares were sterilized by heating in the oven at 160°C for 2 hours, while soil, where necessary, was heat-sterilized in an electric soil sterilizer.

TABLE 4.1

Isolates of Sclerotium rolfsii obtained from different host plants in various localities in northern Nigeria and one locality in southern Nigeria.

Isolate No.	IMI No.	Host plant	Locality
+SR-1	238264	Tomato	Samaru
SR-2	238265	Tomato	Samaru
SR-3	238266	Squash gourd	Kufena
SR-4	// 238267	Groundnut(seed)	Samaru
SR-5	// NA	Potato(tuber)	Zonkwa
SR-6	238269	Onion(bulb)	Samaru
SR-7	NA	Tomato	Bakori
SR-8	238271	Cabbage	Galma
SR-9	// 238272	Cowpea	Dengi
SR-10	238273	Cowpea	Samaru
SR-11	238274	Tomato	Zaria
SR-12	254451	Tomato	Ibadan
SR-13	NA	Pumpkin	Zaria
SR-14	NA	Tomato	Kadawa

+SR = Sclerotium rolfsii

// = Isolates obtained from stock cultures at the Crop Protection Department of Ahmadu Bello University, Zaria, Nigeria.

NA = Not Applicable.

4.1.5 Inoculation and incubation of laboratory cultures.

Inoculation of culture media in these experiments was carried out by placing 5mm mycelial discs taken from the outer margins of 3- to 4-day-old cultures in the centre of a 1- to 2-day-old agar medium in 9.00cm Petri dishes (mycelial part of disc resting on medium). The cultures were then incubated at 30 ± 1°C.

4.1.6 Evaluation of laboratory experiments.

Measurements of mycelial growth of fungal colonies were carried out on solid agar media. Colony diameters were first measured at regular time intervals, along three lines of growth, and the mean calculated after substracting 5mm (diameter of the initial inoculum disc). The radial mycelial growth was then determined by dividing the remaining figure by two to give the amount of mycelial growth in one direction.

All experiments were carried out three times, with four replicates in each treatment. Analysis of variance and other statistical calculations, where necessary, were made by reference to Steel and Torrie (1960).

4.1.7 A comparison of radial growth rate in 14 isolates of Sclerotium rolfsii on PDA.

A comparative study of mycelial growth rate, size, colour of sclerotia and time of initiation of sclerotia in 14 isolates of Sclerotium rolfsii, was made on PDA. Sclerotial size was determined by measuring directly with transparent ruler. Colour of sclerotia was determined with the unaided eye. The number of sclerotia formed per plate by each isolate of the fungus was also assessed by making direct counts in 7-day-old cultures using a Tally counter. At the end of the incubation period, the pH f the PDA on which the fungus was growing was determined by squeezing portions of the agar on pH paper.

4.1.8 Effect of various nutrient media on radial mycelial growth in isolate SR-1 of Sclerotium rolfsii.

The following media were used to evaluate the effect of different media on the radial mycelial growth of isolate SR-1 of S. rolfsii obtained from tomato:

potato dextrose agar, Czapek-Dok agar, nutrient agar, Richard's agar, malt extract agar, corn meal agar, common salt agar and soil extract agar (Appendix A).

The soil extract was prepared according to the method of Bunt and Rovira (1955), by autoclaying 1 kg of garden soil with 1 litre of water for 15 minutes at 1 kg/cm^2 pressure, followed by filteration. Five hundred millilitres of the soil extract were added to a mixture of 500ml of molten agar (17g/l) and other components (Appendix A), boiled for 30 minutes and then autoclaved at 1 kg/cm^2 pressure for 20 minutes.

Other media employed included potato carrot agar, neem (Azadirachta indica A. Juss.) leaf extract agar, mahogany (Khaya senegalensis (Desr.) A. Juss.) seed extract agar, tomato (L. esculentum) stem extract agar, cocoyam (Xanthosoma sp.) petiole extract agar, and carrot (Daucus carota L.) agar. Extracts of these plant materials were prepared as follows: 200g of the appropriate plant part was washed free of sand and plant debris and then cut into pieces. This was boiled in 500 ml distilled water for 15 minutes, in a clean aluminium pot. The extract was strained through fine cheese cloth, and the volume restored to 500ml using distilled water. This volume of extract was then added to 17.0g of agar melted separately in 500ml distilled water to make up to 1 litre before autoclaving.

4.1.9 Effect of pH of PDA on the mycelial growth of Sclerotium rolfsii.

This was determined at various pH values viz: 2, 4, 6, 8, 10, 12 and 14. These values were obtained by using a pH meter which was initially adjusted to pH 7 with a warm buffer solution. Before autoclaving, 200ml of the PDA suspension in cold distilled water contained in 250ml Erlenmeyer flasks was adjusted to the required pH levels. This was achieved by adding the appropriate amounts of 1M concentrated hydrochloric acid (36% purity) or 1M sodium hydroxide solution using a dropping pipettee. Media at different pH values were inoculated with different isolates of S. rolfsii and incubated for 7 days during which observations on mycelial growth and sclerotia formation were made.

4.1.10 Some factors affecting the formation and germination of sclerotia of Sclerotium rolfsii.

Certain physical factors such as wounding and drying were evaluated for their effects on the formation or germination of sclerotia of S. rolfsii on PDA. Sclerotia in seven-day old cultures of the fungus on PDA were used in the germination tests.

4.1.11 Effect of wounding of fungal mycelium on the formation of sclerotia in Sclerotium rolfsii.

Three to four-day old mycelium of S. rolfsii was wounded using a flame-sterilized cork-borer (5mm in diameter). Wounds were made by punching holes (eight per plate) on fungal mycelium growing on PDA. The holes were made at a distance of two to three centimetres from the edge of the Petri dish. Mycelium of S. rolfsii growing on agar medium in control plates was not wounded.

4.1.12 Effect of sun-drying on sclerotial germination in Sclerotium rolfsii.

Sclerotia obtained from naturally infected tomato plants in the field and those grown on PDA in the laboratory were employed in this investigation. The sclerotia were placed in dry Petri dishes and sun-dried for 4 hours (10 a.m. to 2 p.m.) on each of 2 consecutive days. The sclerotia were then plated on PDA (10 per plate) and incubated for 3 days. In control dishes, sclerotia were plated without being previously dried.

4.1.13 Effect of surface-disinfection with 0.5 percent sodium hypochlorite on sclerotial germination in Sclerotium rolfsii.

Two groups of sclerotia (one produced on PDA and the other naturally produced in the field) were each disinfected by immersion in 0.5% sodium hypochlorite solution for two and ten minutes. The sclerotia were then rinsed in three changes of sterile distilled water and dried between sheets of sterile filter paper before plating (10 per dish) on PDA. Sclerotia sown in control plates were treated with sterile distilled water only. All plates were incubated for 3 days.

4.1.14 Effect of wounding of sclerotia of Sclerotium rolfsii on their germination.

Sclerotia (1.00mm in diameter) grown on PDA were wounded by puncturing them with a cool, flame-sterilized needle. The wounds were made gently but firmly so as to pierce the outer rind and cortex of the sclerotia to a depth of approximately 0.1mm. Three punctures were made per sclerotium The sclerotia were then plated (10 per plate) on PDA and incubated for 3 days. Sclerotia in control plates were not punctured.

4.1.15 Study on mycelial incompatibility among various isolates of Sclerotium rolfsii.

Mycelial incompatibility (aversion) was investigated in various isolates of S. rolfsii in this study. Two or three different isolates of the fungus were co-inoculated on the same PDA plate, about 6cm apart. In control plates, two or three mycelial discs from the same isolates of the fungus were similarly co-inoculated. The plates were examined after 3 days of incubation.

4.1.16 Estimation of population density and viability of sclerotia of Sclerotium rolfsii in field soils.

The soils employed in this study were obtained from the Institute for Agricultural Research (IAR) farm complex in Samaru during the dry season (January 31 to March 7, 1981).

4.1.17 Choice of sample areas.

Soil samples for sclerotial population and viability studies were collected from an area 25 x 100m in size in each of 6 fields (Table 4.2) which ranged in size from 3.8 to 5.2 acres. The choice of fields was based on (a) previous occurrence of the basal stem rot disease in the field, (b) the cropping history of the field, for example, fields in which vegetables followed vegetables or cereals were selected.

TABLE 4.2

The size and wet season cropping sequence, over a 5-year period, of six fields in the Institute for Agricultural Research Farm, Samaru, from which soil samples were taken for the study of the population and viability of sclerotia of Sclerotium rolfsii.

		Cropping Sequence*				
Field	Size (Acres)	1976	1977	1978	1979	1980
D1	3.80	G/nut**	Maize	G/nut	Maize	G/nut
D2	3.80	G/nut	Maize	G/nut	Maize	G/nut
D4	4.80	G/nut	G/nut	Fallow	G/nut +Maize	G/nut
BFA	4.30	Tomatoes	Millet	Tomatoes	Tomato	Fallow
U4	5.20	G/nut	Maize	Millet	G/nut	Tomatoes
X2	4.00	Sorghum	G/nut	Cowpea	Tomatoes	Sorghum

* Data supplied by the Agricultural Estate Department, Institute for Agricultural Research, Samaru, Nigeria.

** G/nut = Groundnut

4.1.18 Collection of soil samples.

Field soil samples were collected from 10 sites 10m apart within the prescribed area in each of the six fields selected. Surface litter was first cleared; soil was then collected (using a trowel) from an area 10cm in diameter and up to a depth of 15 cm. Samples from each given field were therefter composited and brought back to the laboratory in separate clean polyethylene bags.

4.1.19 Determination of numbers and viability of sclerotia.

Each of the six soil samples was air-dried by thinly spreading it out on a laboratory bench at room temperatures of 27° to 29°C for 5 days. The soils were then sieved (2mm sieve) ard each sample was assayed for the numbers and viability of any sclerotia of S. rolfsii which they may contain. The methods employed for these tests are described below.

4.1.19.1 The methanol technique.

This was based on the method of Rodriguez-Kabana et al. (1980). The technique was used to determine both the numbers and viability of sclerotia in soil.

In this method, 100g of soil from each field sample was spread evenly over Whatman No. 1 filter paper covering the bottom of a 15-cm-diameter sieve. The soil was evenly moistened with 30ml of 1% (V/V) methanol using a wash bottle. The sieve was then placed in an incubator at 30 ± 1°C for 4 days. Counts of colonies radiating from germinating sclerotia were made at the end of the incubation period.

4.1.19.2 The plant bait method.

This technique was used mainly to assess the viability of sclerotia in field soils. Two types of plant baits were used for this purpose, viz: untreated carrot discs and boiled sorghum seeds (Avizohar-Hershenzon and Shacked, 1968). The sorghum seeds were first boiled for 15 minutes in 5% glucose solution. Each of the six field soil samples was evenly baited in a 25-cm-diameter bowl, using 20 fresh discs of carrot root and 50 boiled sorghum seeds. Each type of bait was mixed separately with 500ml of soil. The soil was adequately moistened with distilled water, and the bowl incubated at room temperature (26° to 29°C) inside a loosely tied polyethylene bag. After 4 days of incubation, the percentage of baits colonized by S. rolfsii was determined as follows: The baited soil was placed on a 2mm sieve and washed under running tap water, then rinsed with

sterile distilled water. Carrot discs or sorghum seeds which remained on the sieve were placed in sterile Petri dishes, lined with moistened filter paper and incubated for two days at room temperature (26° to 29°C). The number of each type of bait colonized was then expressed as a percentage of the total number of that type of bait used.

4.1.20 Laboratory evaluation of some fungicides for their effects on mycelial growth in Sclerotium rolfsii.

Twenty six fungicides, the names, compositions and chemical natures of which are given in Appendix C were evaluated in vitro for activity against S. rolfsii. All fungicides were first evaluated at the following concentrations: 1,000, 500, 250 and 125 parts per million (p.p.m.) of their active ingredients (a.i.). Fungicides which proved active against the pathogen at 125 p.p.m. (a.i.) were further screened at lower concentrations viz. 50, 25, 10 and 5 p.p.m. (a.i.). The method employed for the evaluation of the fungicides was the 'poisoned food technique' (Vyas and Joshi, 1977; Mahmood et al., 1977; Brown and Hendrix, 1980) and is as follows: The appropriate dilution of each fungicide was incorporated into the desired quantity of molten

PDA at 45°C, in 250ml conical flasks, so as to give 100ml of agar - fungicide suspension. The medium was then thoroughly homogenized by gentle circular rotations in order to achieve uniform dispersal of fungicide, and 15 ml of the mixture was poured into sterile 9.00 cm Petri dishes. Control plates contained fungicide-free PDA. The medium was allowed to solidify and after 24 hours, one inoculum disc (5mm in diameter) taken from the edges of 3-day old culture of S. rolfsii was placed in the centre of each Petri dish. Control plates were similarly inoculated and all dishes were incubated at 30 ± 1°C.

The effect of the various fungicides on S. rolfsii was determined by measuring linear growth of the fungus daily along 3 diameters for three days.

4.1.2 Laboratory screening of various fungal isolates for antagonism towards Sclerotium rolfsii.

Sixteen fungi most frequently isolated from tomato plants which were naturally infected by the basal stem rot disease were used in this experiment (the method of isolation of these fungi is described on page 62). These fungal isolates were tested on potato dexrose agar (PDA) for antagonism towards S. rolfsii according to the methods of Butler (1957) and Ghaffar (1966; 1969a):

A single mycelial disc (5mm in diameter) was taken from the edges of 3-to 4-day-old pure culture of the tomato isolate (SR-1) of S. rolfsii and from each of the test fungi, and then inoculated at opposite sides of the same PDA plate, about 70mm apart. The mycelial discs of slow growing fungi such as Fusarium spp. and Aspergillus spp. were placed on the agar plates 2-to 4-days prior to inoculation with S. rolfsii. Control plates were inoculated with S. rolfsii alone. All cultures were incubated at 30 ± 1°C for 7 days.

4.2 Results of laboratory study of aspects of the biology of *Sclerotium rolfsii*.

4.2.1 Microscopic examination of diseased plant materials from the field.

When plant materials naturally infected by *Sclerotium rolfsii* were examined either with the naked eye or with the aid of a dissecting microscope, they revealed conspicuous, white hyphal threads of the pathogen on the affected parts (Plate II).

Numerous sclerotia at different stages of development were also evident in these parts. After such infected materials were incubated in moist polyethylene bags for 3 days at room temperature (25° to 28°C), vigorous and profuse mycelial growth of *S. rolfsii* developed. Other fungi also emerged from incubated tissues, but some of these were soon overgrown by the mycelium of *S. rolfsii*. Only certain greenish colonies of fungi, later identified as *Trichoderma* spp., were not overrun by the pathogen.

Under the dissecting microscope, the surfaces of sclerotia from naturally infested plant materials were found to be smooth and oily. When air-dried, their surfaces became wrinkled. The sclerotia were spherical, oblong or flask-shaped and light-, reddish- or dark-brown

in colour (Appendix B). The size of these sclerotia ranged from 0.3 to 1.2mm, averaging 0.75mm (Appendix B).

4.2.2 Isolation of fungi from diseased plant materials from the field.

Isolation of fungal colonies made from various parts of naturally infected tomato plants consistently yielded S. rolfsii (Plate VIII), in addition to other fungi. The most important fungi isolated from naturally infected tomato plants, or from sclerotia collected from the roots, stems, and fruits of such plants are listed in Tables 4.1 and 4.3. Table 4.3 also shows the relative frequency of occurrence of these fungi.

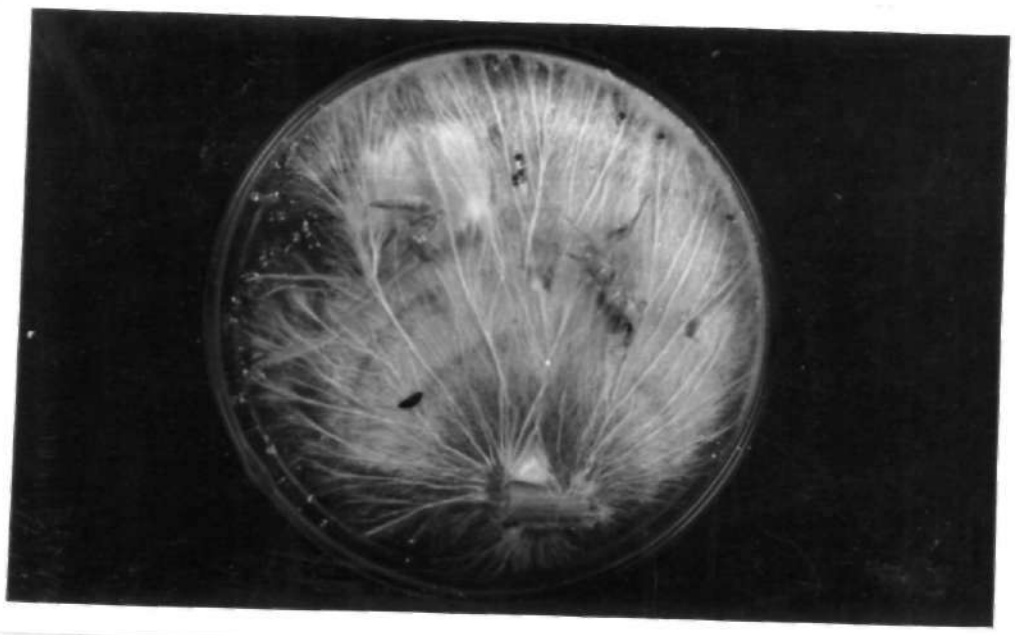


Plate VIII. Characteristic white, ropy mycelium of Sclerotium rolfsii growing on PDA, from a piece of naturally infected tomato stem. (photo taken 5 days after plating)

Table 4.3 (Cont'd)

Nature of plant parts and fungi isolated from them	IMI No.	% frequency of occurrence
Aspergillus sydowii (Bain.&Sartory) Thom & Church	254452	10.0
(D) Symptomless tomato fruits:		
Mucor hiemalis	254465	3.0
Aspergillus tamarii Kita	254466	3.0
Aspergillus sydowii	254463	5.0
(E) Symptomless tomato stems and roots:		
Fusarium sp.	254471	9.0
Mucor hiemalis	-	45.0
Rhizopus sp.	-	30.0
Aspergillus niger	-	30.0
(F) Sclerotia accompanying diseased tomato plants:		
Fusarium oxysporum	254453	15.0
Fusarium equiseti	254464	12.0
Fusarium acuminatum Ell. & Ev.	254462	15.0
Cladosporium cladosporioides (Fres.) de Vries	254476	8.0
Phoma lycopersici Cooke	254459	10.0
Phoma sorghina (Sacc.) Beerema, Derenbosch and van Krest	254474	12.5
Trichoderma harzianum	254478	17.5
Mucor circinelloides van Tieghem	254450	15.0
Mucor hiemalis	254468	15.0

4.2.3 Initiation of sclerotia on PDA by *Sclerotium rolfsii*.

The various isolates of *S. rolfsii* differed widely in the length of time needed for initiation of sclerotia. In most isolates, sclerotial initials were observed (with unaided eye) on PDA in 2 to 4 days. In the squash (SR-3) and Ibadan (SR-12) isolates, formation of sclerotial initials commenced 5 - 7 days after inoculation, while in the tomato (SR-7) isolate, initiation of sclerotia did not occur until 10 to 12 days after inoculation (Table 4.4).

In all cases, the sclerotial initials first appeared as white hyphal knots on the surface of the agar medium. For most isolates, solerotial initials were first observed at the colony margins, after the fungal hyphae reached the edges of the agar medium, or in the centre of the colony around the inoculum disc. In one experiment, however, sclerotial initials were observed in isolate SR-9 before the hyphae reached the edges of the agar medium.

4.2.4 Colour and size of sclerotia on PDA.

Morphologically, mature sclerotia formed by *S. rolfsii* on PDA were similar to those produced naturally by the fungus in the field. In the laboratory, the young

TABLE 4.4

Time of initiation, colour and size of sclerotia in 13 isolates of Sclerotium rolfsii from various locations in northern Nigeria grown on PDA at 30 ± 1°C for 7 days.

S.rolfsii isolate	Some characteristics of sclerotia of S. rolfsii			
	Time(days) of sclerotial initiation	Colour	Mean No/Plate	Mean size(mm)
SR-1	4 - 5	reddish-brown	455^{de}	0.91 ± 0.24
SR-2	4 - 5	reddish-brown	529.8^{e}	0.91 ± 0.19
SR-3	6 - 7	reddish-brown	85.5^{a}	0.79 ± 0.21
SR-4	3 - 4	dark-brown	343.6^{c}	1.00 ± 0.21
SR-5	2 - 4	dark-brown	229.5^{b}	1.00 ± 0.17
SR-6	2 - 4	dark-brown	377.5^{c}	0.94 ± 0.19
SR-7	10 - 12	dark-brown	34.0^{a}	0.88 ± 0.15
SR-8	3 - 4	dark-brown	524.8^{e}	1.00 ± 0.19
SR-9	3 - 4	dark-brown	480.2^{de}	0.94 ± 0.20
SR-10	3 - 4	dark-brown	168.7^{b}	1.00 ± 0.11
+SR-12	7 - 8	dark-brown	70.2^{a}	2.50 ± 0.85
SR-13	3 - 4	dark-brown	199.3^{b}	0.95 ± 0.75
SR-14	3 - 4	dark-brown	431.0^{cd}	0.90 ± 0.63

+ = Isolate from Ibadan (southern Nigeria).

Means in a column followed by the same letter are not significantly different (P = 0.05).

sclerotia passed through various shades of colour from white to light yellow and finally to the brownish colour which characterised mature sclerotia in this study. Of the 13 isolates of S. rolfsii investigated (one isolate was lost) three isolates viz: SR-1, SR-2. and SR-3 produced sclerotia which were reddish-brown at maturity while the other 10 isolates formed sclerotia which were dark-brown in colour (Table 4.4).

Not all the white sclerotial initials matured on PDA during the 14-day incubation period in this study. In some isolates, the white initials remained so until 4 weeks after formation. However, a high percentage (92 to 100%) of mature sclerotia was usually recorded for most isolates. On PDA, isolates of S. rolfsii from various locations in the savanna zones of northern Nigeria produced sclerotia which ranged from 0.5 to 2.0mm in diameter and averaging 0.79mm to 1.00mm (Table 4.4). In contrast, isolate SR-12 obtained from Ibadan in the rainforest zone of Nigeria formed larger sclerotia of diameter 2.0 to 3.5mm averaging 2.5mm.

4.2.5 Distribution of sclerotia on PDA.

The pattern of distribution of sclerotia on the surface of agar medium varied in different isolates of S. rolfsii and also within a given isolate of the fungus.

Sclerotia were usually formed in rhythmic concentric zones in most isolates. In others, however, sclerotia were scattered over the agar surface (Plate IX).

Mature sclerotia either lay singly on fungal hyphae, or were clumped together in groups of two or more sclerotia, which coalesced to form irregular structures similar to those observed in the field. After sclerotia were detached from the hyphae bearing them, the point of attachment of each sclerotium to the hypha remained visible as a short white stalk on the under surface of the sclerotium.

4.2.6 Hyphal and sclerotial exudation.

The hyphae and sclerotia in all isolates of S. rolfsii studied produced white glittering, watery exudate on PDA plates. This exudate was usually observed 2-to 3-days after inoculation, and persisted throughout the 14-day period of observation in this investigation. It was found (using pH papers) to be acidic with pH values of 4.0 to 4.3. At the end of the incubation period, the PDA itself on which S. rolfsii was growing had also become strongly acidic with pH values of 2.0 to 3.0. The PDA had an initial pH value of 5.6.

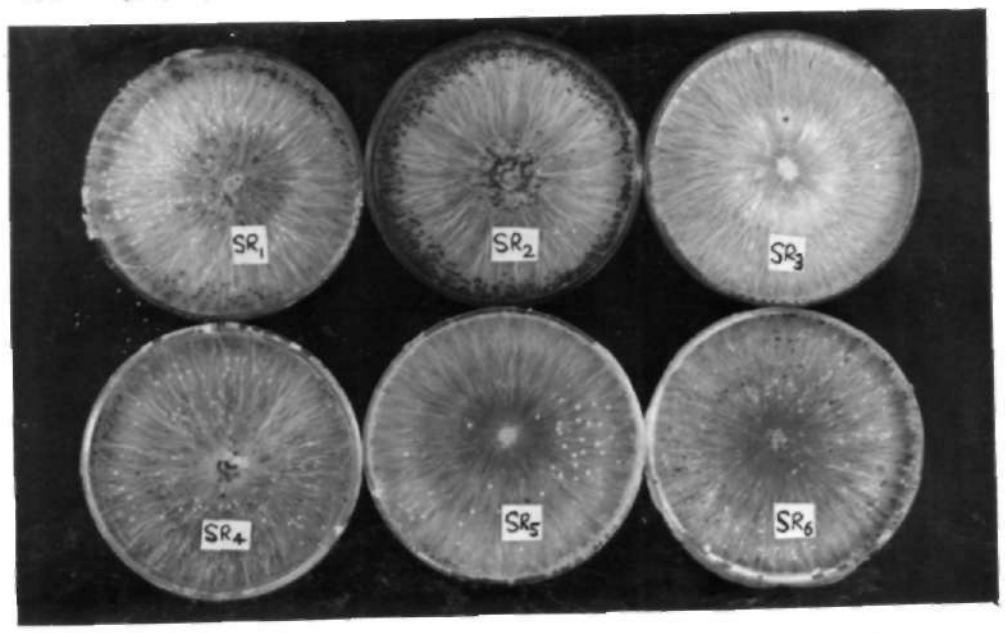


Plate IX. Distribution of sclerotia of Sclerotium rolfsii on PDA in concentric pattern (SR-2) and scattered pattern (SR-1, SR-3, SR-4, SR-5, SR-6).

4.2.7 A comparison of radial mycelial growth rate in 14 isolates of Sclerotium rolfsii on PDA.

All the 14 isolates of S. rolfsii tested in this study grew fairly rapidly, covering 9.00cm Petri dishes in 3 to 4 days. However, there were differences between the growth rates of mycelia of the different isolates. The growth rate of mycelium of isolate SR-12 was significantly lower (P = 0.05) than the growth rates in some isolates of the fungus on the first, second and third days after PDA plates were seeded (Table 4.5). Several isolates did not show a significant difference in growth rate up to the third day. However, growth rate on the third day of incubation in two isolates viz: SR-9 and SR-12 were significantly lower than in most of the other isolates. Isolate SR-12 of the fungus showed the least growth throughout the period of incubation, and on the third day, differences in growth between this isolate and the other 12 isolates were significant (P = 0.05).

TABLE 4.5

Radial mycelial growth rate of 14 isolates of *Sclerotium rolfsii* obtained from various host plants from different localities in northern Nigeria and one locality in southern Nigeria and grown on PDA at 30 ± 1°C for 3 days

Isolates	Mean radial growth (mm) after: 24hrs	48hrs	72hrs
1. SR-1 (Isolated from tomato from Samaru)	9.3	24.2	41.9
2. SR-2 (Isolated from tomato from Samaru)	9.9	25.8	42.3
3. SR-3 (Isolated from squash gourd from Kufena)	8.3	23.4	41.8
4. SR-4 (Isolated from groundnut seed from Samaru)	7.7	23.0	42.1
5. SR-5 (Isolated from potato tuber from Zonkwa)	7.7	23.0	42.4
6. SR-6 (Isolated from onion bulb from Samaru)	7.0	23.3	42.4
7. SR-7 (Isolated from tomato from Bakori)	8.5	22.3	40.7
8. SR-8 (Isolated from cabbage from Galma)	7.8	25.7	42.5
9. SR-9 (Isolated from cowpea from Dengi)	7.0	21.9	38.2
10. SR-10(Isolated from cowpea from Samaru)	6.8	22.1	39.6
11. SR-12(Isolated from tomato from Zaria)	5.5	20.3	35.6
12. SR-13(Isolated from tomato from Ibadan)	7.6	25.1	41.3
13. SR-14(Isolated from pumpkin from Zaria)	8.9	25.0	42.3
14. SR-15(Isolated from tomato from Kadawa)	8.9	25.0	42.2
L. S.D. (P = 0.05)	2.97	4.39	2.77

4.2.8 Effect of various nutrient media on radial mycelial growth in isolate SR-1 of *Sclerotium rolfsii*.

Data in Table 4.6 show that the media which best supported the mycelial growth of *S. rolfsii*, appeared to be potato dextrose agar, potato carrot agar, malt extract agar, mahogany seed extract agar, and tomato stem extract agar. They all supported growth about equally well. On the third day, there was no significant difference ($P = 0.05$) in the mycelial growth rate among these media. The poorest growth throughout the experiment was recorded with cocoyam petiole extract agar whose growth supporting ability was, however, not significantly different from those of Czaper-Dox agar, nutrient agar and Richard's agar on the third day after inoculation. The other media employed took a middle course with or without significant differences in their abilities to support the mycelial growth of *S. rolfsii*.

TABLE 4.6

Effect of 14 different solid media on the radial mycelial growth in isolate SR-1 of *Sclerotium rolfsii*, incubated at 30 ± 1°C for 3 days.

	Media	Mean radial mycelial growth (mm) after:		
		24hrs	48hrs	72hrs
1.	Potato dextrose agar	10.3	28.5	42.5
2.	Potato carrot agar	12.2	25.9	42.5
3.	Czaper-Dox agar	11.9	18.0	21.7
4.	Neem leaf extract agar	5.8	14.2	28.4
5.	Nutrient agar	8.3	16.0	24.8
6.	Corn meal agar	12.7	22.7	33.5
7.	Malt extract agar	12.4	27.4	42.5
8.	Mahogany seed extract agar	9.7	28.3	42.5
9.	Tomato stem extract agar	12.5	36.3	42.5
10.	Common salt agar	12.0	29.0	41.1
11.	Richard's agar	5.2	10.9	24.2
12.	Cocoyam petiole extract agar	1.1	6.5	21.1
13.	Soil extract agar	8.0	21.6	36.5
14.	Carrot agar	8.2	24.4	41.6
	L. S. D. (P = 0.05)	2.06	3.67	8.76

4.2.9 Effect of pH of PDA on the mycelial growth of Sclerotium rolfsii.

The results (Table 4.7) show that mycelial growth occurred in isolate SR-1 of S. rolfsii at all pH levels tested, except pH 14. Although the best mycelial growth for this isolate occurred between pH 4 and pH 6, the analysis of variance indicate that growth within this pH range was not significantly different (P = 0.05) from that at pH 2 or pH 12. It was also observed that the length of time required for initiation of sclerotia did not appear to have been affected by the pH of the medium. The levels of pH which permitted mycelial growth also allowed large numbers of sclerotia to be formed.

Following the observation that isolate SR-1 of S. rolfsii failed to grow at pH 14, eleven isolates of the fungus used in this investigation were grown at this pH level. The purpose was to determine whether any other isolates of the pathogen could fail to grow at pH 14.

Table 4.8 shows that seven of the isolates grew at pH 14 with the mean radial growth ranging from 1.4mm to 25.1mm per day. Mycelial growth in isolate SR-14 was significantly (P = 0.05) lower than in the other

six isolates, namely: SR-2, SR-4, SR-6, SR-8, SR-9 and SR-13. Slight differences in the length of time of initiation of sclerotia appeared to have occurred among the isolates.

TABLE 4.7

Effect of pH of potato dextrose agar on radial mycelial growth, time required for initiation and amount of sclerotia formed by isolate SR-1 of *Sclerotium rolfsii* incubated at 30 ± 1°C for 7 days.

pH of medium	Mean radial mycelial growth (mm) per day	Time (days) of sclerotia initiation	Amount of sclerotia formed*
5.6(Normal PDA, control)	26.7	3	+++
2	25.5	4	+++
4	27.0	4	+++
6	27.9	4	+++
8	25.7	4	+++
10	14.5	4	+++
12	15.0	4	+++
14	0.0	-	Nil
L.S.D (0.05)	16.0	ND	ND

* + = 1 to 100 sclerotia formed per plate
++ = 101 to 200 sclerotia formed per plate
+++ = 201 sclerotia and above formed per plate
ND = Not determined.

TABLE 4.8

Effect of potato dextrose agar at pH 14 on radial mycelial growth, time required for initiation and amount of sclerotia formed by various isolates of *Sclerotium rolfii* incubated at 30 ± 1°C for 7 days.

Isolate of *S. rolfsii*	Mean radial mycelial growth (mm) per day	Time (days) of sclerotia initiation	Amount of sclerotia formed*
SR-1	0.0	NA	Nil
SR-2	12.9	4	+++
SR-3	0.0	NA	Nil
SR-4	16.3	4	+++
SR-6	25.1	4	+++
SR-8	18.0	4	+++
SR-9	17.5	4	+++
SR-10	0.0	NA	Nil
SR-12	0.0	NA	Nil
SR-13	13.4	5	++
SR-14	1.4	5	+++
L.S.D. (0.05)	9.0	ND	ND

* = 1 to 100 sclerotia formed per plate
++ = 101 to 200 sclerotia formed per plate
+++ = 201 sclerotia and above formed per plate
ND = Not determined.

4.2.10 Some factors affecting the formation and germination of sclerotia in Sclerotium rolfsii.

4.2.10.1 Effect of wounding of fungal mycelium on the formation of sclerotia in Sclerotium rolfsii.

Numerous sclerotial initials appeared on wounded mycelium, especially along the cutting lines, within 24 hours after wounding, while mycelia in unwounded control plates normally produced sclerotial initials in 2 to 12 days, depending on the isolate of the fungus. Wounds induced a similar behaviour in all isolates of S. rolfsii tested in this study (Plate X).

Wounded young mycelia produced more sclerotia than unwounded mycelia of the same age and about 18 to 50% of the sclerotia produced by wounded mycelium of S. rolfsii were formed on or around the points of damage. In older cultures (4 to 5 days old), wounding the mycelium was less effective in inducing sclerotial formation or increasing the total number of sclerotia produced by the fungus.

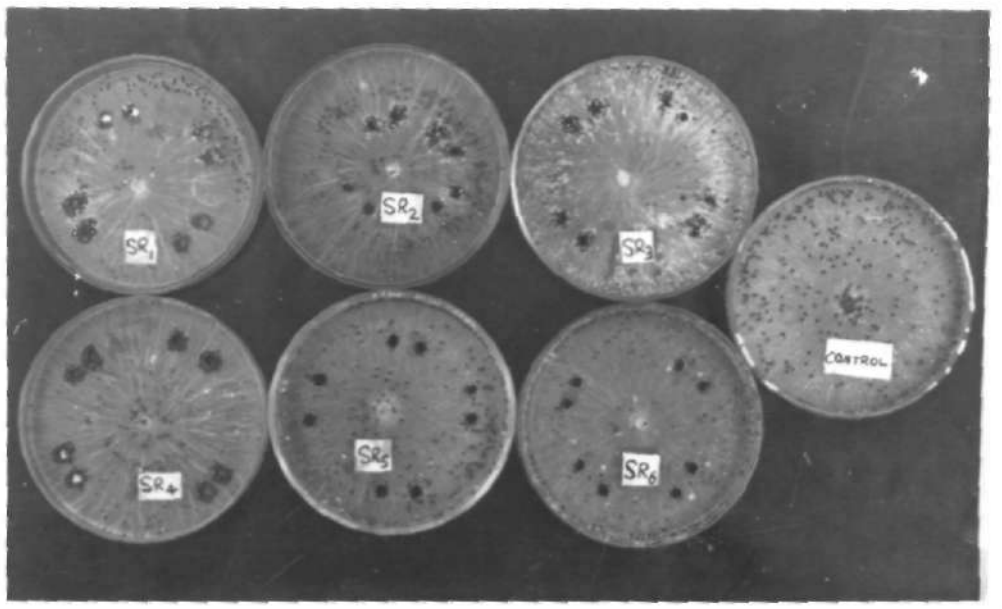


Plate X. Wounded young mycelium of Sclerotium rolfsii on PDA with sclerotia clustered around wound sites in plates containing wounded mycelia of isolates SR-1 to SR-6 and scattered in unwounded control plate.

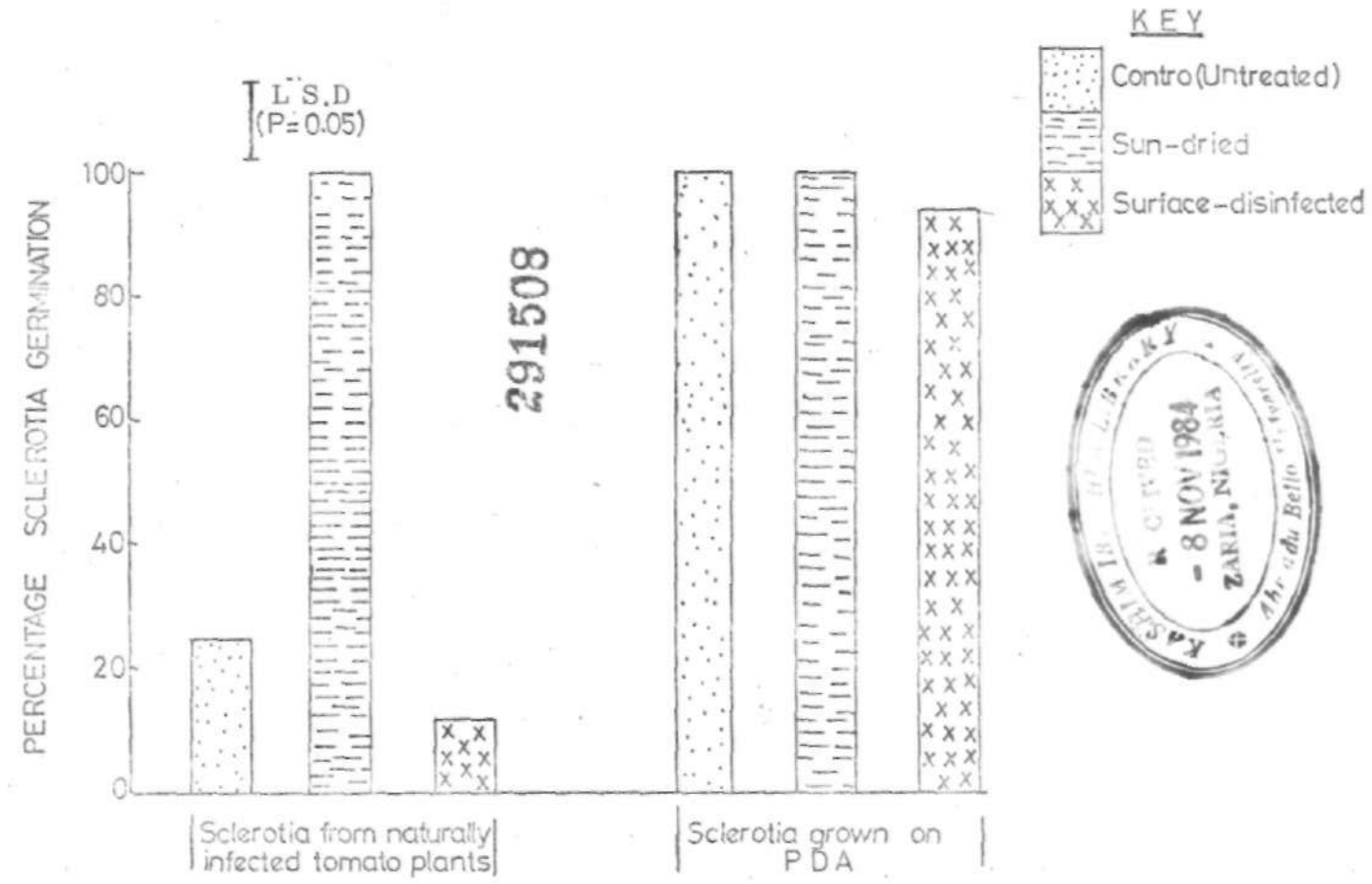
4.2.10.2 Effect of sun-drying on sclerotial germination in Sclerotium rolfsii.

In pre-liminary observations during the wet season, it was noticed that when sclerotia were exposed to air at room temperature (25° to 28°C) in dry Petri dishes, their surfaces quickly became wrinkled and the colour darkened. The original state of the sclerotia was, however, rapidly restored when they were placed on a damp filter paper. This change was more remarkable in sclerotia obtained from naturally infected tomato plants in the field than in those grown on PDA in the laboratory.

The results in Figure 2 show a significant difference (P = 0.05) in germination between sun-dried and non-dried sclerotia obtained from tomato plants naturally infected by S. rolfsii in the field. The difference between similarly treated, but PDA-grown sclerotia was not significant (P = 0.05).

4.2.10.3 Effect of surface disinfection with 0.5 percent sodium hypochlorite solution on sclerotial germination in Sclerotium rolfsii.

Surface disinfection with 0.5% sodium hypochlorite for 2 minutes did not significantly affect the germination of sclerotia of S. rolfsii (Fig. 2). This was irrespective



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Fig. 2: Effect of sun-drying and surface-disinfection with 0.5% Sodium hypochlorite solution for 2 minutes on percentage germination of sclerotia of Sclerotium rolfsii grown naturally on rain-fed tomato plants and on PDA and in cubated at 30 ± 1°C

of whether the sclerotia were obtained from naturally infected plants or from artificially inoculated PDA culture.

Immersion of sclerotia in 0.5% sodium hypochlorite solution for more than 2 minutes and up to 10 minutes completely decolorised the sclerotia to a white colour and rendered them unviable.

4.2.10.4 Effect of wounding of sclerotia of Sclerotium rolfsii on their germination.

All the wounded 40 sclerotia and the non-wounded 20 sclerotia in control plates germinated 24 hours after plating. There was no significant difference (P = 0.05) between the two treatments. However, the further growth of fungal hyphae was more rapid and more profuse in wounded than in intact sclerotia.

4.2.10.5 Mycelial incompatibility among various isolates of Sclerotium rolfsii.

Results presented in Table 4.9 show that mycelial incompatibility or aversion occurred between some isolates of S. rolfsii, but not between others. Aversion was commonly observed only between isolates of the fungus from different hosts. In isolates of S. rolfsii showing aversion or incompatibility, the mycelia of the two or

TABLE 4.9

In vitro occurrence of mycelial incompatibility between different isolates of Sclerotium rolfsii obtained from various locations in northern Nigeria.

Test isolate	Locality where obtained	Isolates showing incompatibility (aversion)	Isolates showing compatibility (non-aversion)
SR-1	Samaru	SR-3,SR-4,SR-5,SR-6	SR-1,SR-2,SR-9
SR-2	Samaru	SR-4,SR-3,SR-5,SR-6	SR-1,SR-2,SR-9
SR-3	Kufena	SR-1,SR-2,SR-4,SR-5	SR-3
SR-4	Samaru	SR-1,SR-2,SR-3,SR-6	SR-4
SR-5	Zonkwa	SR-1,SR-2,SR-3,SR-4	SR-5,SR-6
SR-6	Samaru	SR-1,SR-2,SR-3,SR-4	SR-5,SR-6
SR-8	Galma	SR-12,SR-13	-
SR-9	Dengi	SR-4,SR-6,SR-13	SR-9,SR-10,SR-12
SR-10	Samaru	SR-1,SR-2,SR-12	-
SR-12*	Ibadan	SR-13	SR-12
SR-13	Zaria	SR-14	SR-5,SR-6,SR-13
SR-14	Kadawa	SR-4,SR-9,SR-12	SR-2,SR-9,SR-14

*SR-12 = Isolate from Ibadan, western Nigeria.

more colonies did not intermingle on PDA plates, but remained separated by a clear zone of demarcation, 1 to 3mm wide (Plate XI). In such isolates, sclerotia were formed by each isolate of the fungus on its own side of the plate, including the areas bordering on the zones of inhibition between two or more colonies.

In isolates which showed mycelial compatibility, or non-aversion, there was a complete intermingling of mycelia of the different isolates where the colony margins met, so that the interfaces between the colonies were almost indistinguishable (Plate XI). Sclerotia were formed in all parts of such colonies including the meeting points. Mycelial compatibility was recorded only between isolates of the fungus from the same host plant.

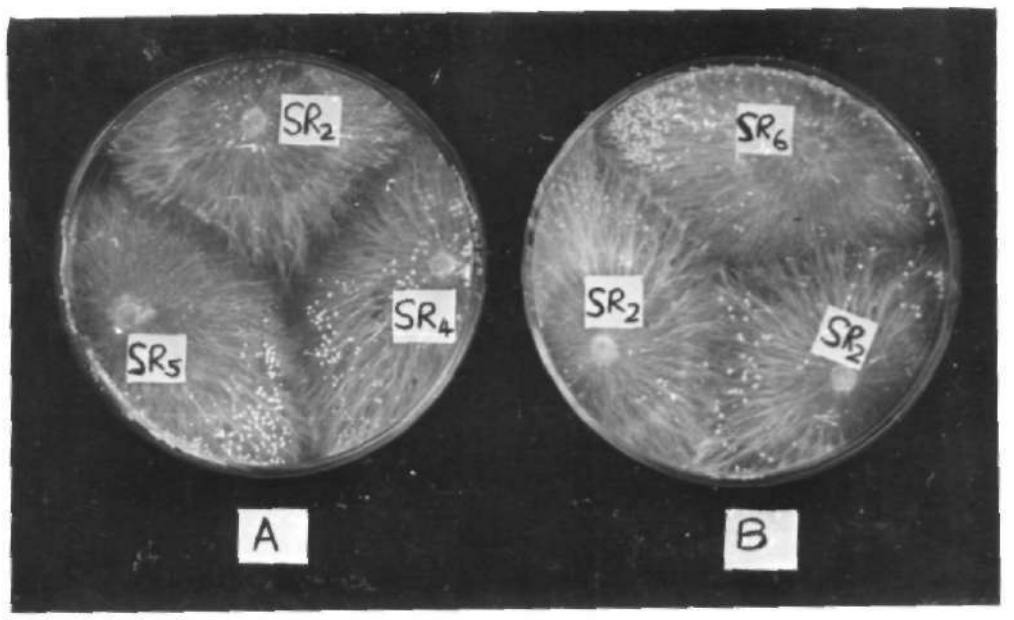


Plate XI. (A) Isolates SR-2, SR-4 and SR-5 of Sclerotium rolfsii from different host plants displaying mycelial incompatibility (aversion) on PDA with clear areas of demarcation between the isolates.

(B) Two colonies of isolate SR-2 of Sclerotium rolfsii from the same host plant (tomato) showing mycelial compatibility (non-aversion) with each other and with isolate SR-6 (from onion) on PDA.

4.2.12 Estimation of population density and viability of sclerotia of Sclerotium rolfsii in field soils.

Results in Table 4.10 show that the population of viable sclerotia in the various field soils ranged from 2.70 to 9.60 sclerotia per 100g of soil. The differences between viable sclerotial population of some of the fields were found to be significant (P = 0.05). The highest numbers of viable sclerotia were recorded in soils from Botany Field 'A' (BFA) and field U4.

Data in Table 4.11 indicate that a fairly high percentage (33 to 50%) of both carrot root discs and sorghum seed baits were colonized by S. rolfsii in soils from fields BFA and U4. Differences in the number of colonized baits in some field soils were significant (P = 0.05). But there was no appreciable difference between percentage colonization of carrot discs and of sorghum seeds by S. rolfsii.

TABLE 4.10

Number of viable sclerotia of *Sclerotium rolfsii* in soil from fields in Samaru in 1981 dry season.

Fields	Crops grown in two previous years	Mean No. of sclerotia/100g of soil*
D1	Maize, groundnut	3.30
D2	Maize, groundnut	4.30
D4	groundnut, groundnut	2.70
BFA	tomato, fallow	9.00
U4	groundnut, tomato	9.60
X2	tomato, sorghum	3.70
L.S.D. (P=0.05)		1.30

*Mean of three experiments.

TABLE 4.11

Colonization of plant tissue baits by *Sclerotium rolfsii* in Samaru soils in 1981 dry season.

Fields	Crops grown in two previous years	% colonization*	
		Carrot root	Sorghum seed
D1	maize, groundnut	2.0	3.3
D2	maize, groundnut	5.6	7.6
D4	groundnut, groundnut	4.7	3.7
BFA	tomato, fallow	45.0	50.0
U4	groundnut, tomato	33.3	35.0
X2	tomato, sorghum	5.0	7.0
L.S.D. (P=0.05)		2.45	3.46

*Mean of three experiments.

4.2.13 Laboratory evaluation of some fungicides for their effects on mycelial growth of Sclerotium rolfsii.

The results presented in Table 4.12 show that 13 fungicides completely suppressed mycelial growth in S. rolfsii at concentrations of 125 to 1,000 p.p.m. (a.i.). These fungicides were: Calixin M, Vitavax 75, Vitavax 200, Vitavax 300, Delsene M, Dithane M45, Bayleton, Verdasan, Ceresan, Aldrex T, Aatopam-N, captan and captafol. Four other fungicides, namely: Plantvax 75, Du-Ter 50, PCNB and Fernasan D severely reduced mycelial growth at the same concentrations while Benlate and zineb had similar effects only at 1,000 ppm (Plate XII). Benlate and Polyram Combi gave a moderate inhibitory action only at higher concentrations (500 - 1,000 p.p.m. (a.i.)).

The other fungicides evaluated at 125 to 1,000 p.p.m. (a.i.) showed little or no inhibitory action against the fungus and consequently were not tested at lower concentrations. These fungicides were: Bavistin, Tecto, Maneb, Calixin, Ridomil, zineb and Topsin M.

Data in Table 4.13 indicate that Vitavax 75 and Ceresan completely prevented mycelial growth in S. rolfsii at concentrations of 5 to 50 p.p.m. (a.i.). Similar effects were exhibited by Vitavax 200, Vitavax 300, and

Verdasan. Captafol and Du-Ter 50 at 50 p.p.m. (a.i.), PCNB at 25 to 50 p.p.m. (a.i.) drastically reduced mycelial growth in S. rolfsii. Eight fungicides showed no appreciable action against S. rolfsii at 5 to 50ppm (a.i.). These fungicides were: Dithane M45, Bayleton, captan, Calixin M, Plantvax 75, Delsene M, Aldrex T and Aatopam-N. The following fungicides were ineffective at the concentrations (a.i.) indicated: Du-Ter 50, captafol and Verdasan (5 to 25 p.p.m.) and PCNB (5 to 10 p.p.m.(a.i).

In all cases in which suppression of mycelial growth was not complete, it was found that inhibitory action by the fungicides normally increased with increasing concentration of the fungicides.

TABLE 4.12

Effect of type and concentration of fungicides on mycelial growth of isolate SR-1 of *Sclerotium rolfsii* on PDA after 3 days at 30 ± 1°C.

Fungicide	Active ingredient concentration (p.p.m.) of fungicide in agar	Mycelial Growth of *S. rolfsii*	
		Colony diameter (cm)	Growth as % of growth of control
Control	No fungicide	8.5	100.0
Calixin M	1,000	0.0	0.0
	500	0.0	0.0
	250	0.0	0.0
	125	0.0	0.0
Bavistin	1,000	7.5	88.2
	500	8.1	95.3
	250	8.1	95.3
	125	8.5	100.0
Polyram Combi	1,000	1.2	14.1
	500	2.7	31.8
	250	2.7	31.8
	125	6.1	71.8
Vitavax 75	1,000	0.0	0.0
	500	0.0	0.0
	250	0.0	0.0
	125	0.0	0.0

Table 4.12 (cont'd)

Fungicide	Active ingredient concentration(p.p.m) of fungicide in agar	Mycelial Growth of S. rolfsii	
		Colony diameter(cm)	Growth as % of growth of control
Vitavax 200	1,000	0.0	0.0
	500	0.0	0.0
	250	0.0	0.0
	125	0.0	0.0
Vitavax 300	1,000	0.0	0.0
	500	0.0	0.0
	250	0.0	0.0
	125	0.0	0.0
Tecto	1,000	4.4	51.8
	500	5.4	63.5
	250	7.3	85.9
	125	7.6	89.4
Delsene M	1,000	0.0	0.0
	500	0.0	0.0
	250	0.0	0.0
	125	0.0	0.0
Dithane M45	1,000	0.0	0.0
	500	0.0	0.0
	250	0.0	0.0
	125	0.0	0.0

Table 4.12 (cont'd)

Fungicide	Active ingredient concentration (p.p.m.) of fungicide in agar	Mycelial growth of S. rolfsii	
		Colony diameter(cm)	Growth as % of growth of control
Bayleton	1,000	0.0	0.0
	500	0.0	0.0
	250	0.0	0.0
	125	0.0	0.0
Benlate	1,000	0.2	2.4
	500	1.5	17.6
	250	3.5	41.2
	125	5.7	67.1
Du-Ter 50	1,000	0.1	1.2
	500	0.2	2.5
	250	0.2	2.4
	125	0.2	2.4
PCNB	1,000	0.4	4.7
	500	0.6	7.1
	250	0.6	7.1
	125	0.6	7.1
Verdasan	1,000	0.0	0.0
	500	0.0	0.0
	250	0.0	0.0
	125	0.0	0.0

Table 4.12 (cont'd)

Fungicide	Active ingredient concentration (p.p.m.) of fungicide in agar	Mycelial growth of S. rolfsii	
		Colony diameter(cm)	Growth as % of growth of control
Fernasan D	1,000	0.1	1.2
	500	0.1	1.2
	250	0.4	4.7
	125	0.6	7.1
Ceresan	1,000	0.0	0.0
	500	0.0	0.0
	250	0.0	0.0
	125	0.0	0.0
Aldrex T	1,000	0.0	0.0
	500	0.0	0.0
	250	0.0	0.0
	125	0.0	0.0
Zineb	1,000	0.5	5.9
	500	3.0	35.3
	250	5.2	61.2
	125	6.9	81.2
Captafol	1,000	0.0	0.0
	500	0.0	0.0
	250	0.0	0.0
	125	0.0	0.0

Table 4.12 (cont'd)

Fungicide	Active ingredient concentration (p.p.m.) of fungicide in agar	Mycelial growth of S. rolfsii	
		Colony diameter(cm)	Growth as % of growth of control
Topsin M	1,000	6.7	78.8
	500	7.0	82.4
	250	7.5	88.2
	125	7.6	89.4
Maneb	1,000	2.9	34.1
	500	6.1	71.8
	250	6.3	74.1
	125	7.3	85.9
Calixin	1,000	2.7	31.8
	500	5.0	58.8
	250	7.1	83.5
	125	7.7	90.6
Aatopam-N	1,000	0.0	0.0
	500	0.0	0.0
	250	0.0	0.0
	125	0.0	0.0
Captan	1,000	0.0	0.0
	500	0.0	0.0
	250	0.0	0.0
	125	0.0	0.0
Plantvax 75	1,000	0.0	0.0
	500	0.0	0.0
	250	0.4	4.7
	125	0.6	7.1
Ridomil	1,000	1.8	21.2
	500	5.2	61.2
	250	5.8	68.2
	125	7.5	88.2

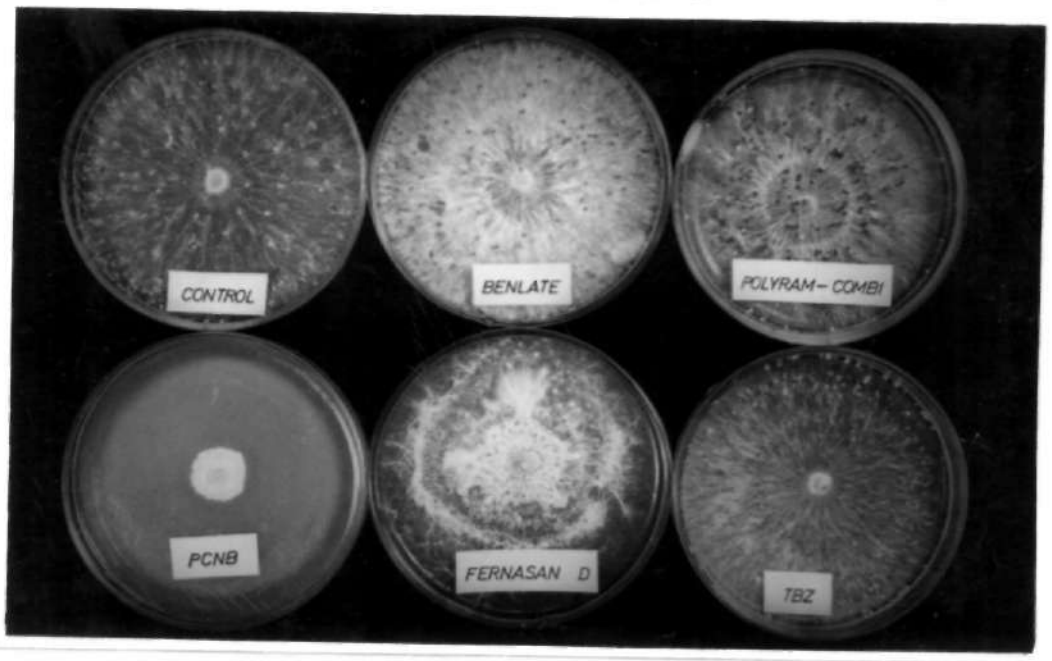


Plate XII. Effect of various fungicides (at 125 p.p.m. a.i.) on mycelial growth and sclerotia production in Sclerotium rolfsii, 7 days after inoculation on PDA.

TABLE 4.13

Effect of type and concentration of fungicides on mycelial growth of isolate SR-1 of *Sclerotium rolfsii* on PDA after 3 days at 30 ± 1°C.

Fungicide	Active ingredient concentration (p.p.m.) of fungicide in agar	Mycelial growth of *S. rolfsii*	
		Colony diameter(cm)	Growth as % of growth of control
Control	No fungicide	8.5	100.0
Vitavax 75	50	0.0	0.0
	25	0.0	0.0
	10	0.0	0.0
	5	0.0	0.0
Vitavax 200	50	0.0	0.0
	25	0.0	0.0
	10	0.0	0.0
	5	3.0	35.2
Vitavax 300	50	0.0	0.0
	25	0.3	3.5
	10	0.4	4.7
	5	0.9	10.6
Dithane M45	50	7.1	83.5
	25	7.4	87.1
	10	7.8	91.8
	5	7.9	92.9

Table 4.13 (cont'd)

Fungicide	Active ingredient concentration (p.p.m.) of fungicide in agar	Mycelial growth of S. rolfsii	
		Colony diameter(cm)	Growth as % of growth of control
Bayleton	50	3.4	40.0
	25	4.7	55.3
	10	6.7	78.8
	5	7.0	82.4
Captan	50	8.5	100.0
	25	8.5	100.0
	10	8.5	100.0
	5	8.5	100.0
Calixin M	50	3.7	43.5
	25	7.0	82.4
	10	7.6	89.4
	5	7.7	90.6
Plantvax 75	50	6.4	75.3
	25	7.3	85.9
	10	7.9	92.9
	5	8.5	100.0
Du-Ter 50	50	0.4	4.7
	25	1.4	16.5
	10	3.5	41.2
	5	4.1	48.2

Table 4.13 (cont'd)

Fungicide	Active ingredient concentration (p.p.m.) of fungicide in agar	Mycelial growth of S. rolfsii	
		Colony diameter(cm)	Growth as % of growth of control
Ceresan	50	0.0	0.0
	25	0.0	0.0
	10	0.0	0.0
	5	0.0	0.0
Captafol	50	0.9	10.6
	25	1.8	21.2
	10	3.6	42.4
	5	3.7	43.5
PCNB	50	0.7	8.2
	25	0.7	8.2
	10	4.2	49.4
	5	4.5	52.9
Delsene M	50	8.5	100.0
	25	8.5	100.0
	10	8.5	100.0
	5	8.5	100.0
Aldrex T	50	4.4	51.8
	25	5.4	63.5
	10	7.2	84.7
	5	7.5	88.2
Verdasan	50	0.0	0.0
	25	1.6	18.8
	10	3.6	42.4
	5	7.1	83.5
Aatopam-N	50	4.5	52.9
	25	6.6	77.6
	10	7.2	84.7
	5	7.5	88.2

4.2.13.1 Effect of various fungicides on mycelial growth in different isolates of Sclerotium rolfsii.

Results in Table 4.14 show that six of the 16 fungicides at a concentration of 125 ppm active ingredient completely inhibited mycelial growth in all 11 isolates of S. rolfsii tested. These fungicides were: Ceresan, Vitavax 75, Vitavax 200, Vitavax 300, Bayleton and Verdasan. Nine fungicides namely: Aatopam-N, Aldrex T, Du-Ter 50, captan, Plantvax 75, Dithane M45, Calixin M, Delsene M and captafol completely suppressed mycelial growth in some isolates of the fungus but not in others.

4.2.13.2 Effect of some fungicides on the germination of sclerotia of isolate SR-1 of Sclerotium rolfsii.

It was found that Vitavax 75 and Vitavax 300 at 125 ppm (a.i.) completely prevented sclerotial germination of isolate SR-1 of S. rolfsii (Table 4.15). There was, however, no significant difference (P=0.05) between the effects of these two fungicides and that of Verdasan. The results for Vitavax 75, Vitavax 300 and Verdasan were significantly (P=0.05) different from those obtained for other fungicides. Nine fungicides viz: Aatopam-N, PCNB, Aldrex T, captan, Plantvax 75, Dithane M45, Calixin M, Delsene M and captafol showed no inhibitory effects whatsoever at 125 ppm (a.i.) towards the germination of sclerotia in isolate SR-1 of the fungus.

TABLE 4.14

Effect of various fungicides at a concentration of 125 p.p.m. (a.i.) on mycelial growth in different isolates of Sclerotium rolfsii after incubation for 3 days at 30 ± 1°C.

Fungicide	Colony diameter** (cm)										
	Isolates of Sclerotium rolfsii										
	SR-1	SR-2	SR-3	SR-4	SR-6	SR-7	SR-8	SR-9	SR -10	SR-12	SR-13
Control (no fungicide)	8.3	8.4	8.3	8.4	8.4	8.1	8.5	7.6	7.9	7.1	8.2
Aatopam-N	0.0	6.4	0.0	0.0	4.1	0.0	0.0	*NI	0.0	0.0	0.0
PCNB	2.1	3.2	1.5	1.3	1.3	1.9	2.7	1.9	1.4	3.1	1.3
Aldrex T	0.0	6.5	NI	8.4	5.9	NI	NI	4.7	7.9	7.1	8.2
Du-Ter 50	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Captan	0.0	8.2	6.1	8.4	5.4	0.0	0.0	7.9	6.2	5.0	0.0
Plantvax 75	0.5	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dithane M-45	0.0	8.4	0.0	8.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Calixin M	0.0	7.5	0.0	0.0	0.0	7.7	0.0	0.0	0.0	7.1	0.0
Delsene M	0.0	8.4	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0
Captafol	0.0	1.9	1.7	3.8	2.4	0.0	4.3	0.0	0.0	0.0	2.6

*NI = Not Investigated.

** = Ceresan, Vitavax 75, Vitavax 200, Vitavax 300, Bayleton and Verdasan completely inhibited mycelial growth in all isolates of S. rolfsii tested at 125 p.p.m. (a.i.)

TABLE 4.15

Effect of some fungicides at a concentration of 125 p.p.m. (a.i.) on the germination of sclerotia of isolate SR-1 of *Sclerotium rolfsii* after 3 days incubation at 30 ± 1°C.

Fungicide	Mean* % germination of sclerotia
Control (no fungicide)	100.0[a]
Aatopam-N	100.0[a]
PCNB	100.0[a]
Aldrex T	100.0[a]
Ceresan	62.5[c]
Vitavax 75	0.0[e]
Vitavax 200	52.5[c]
Vitavax 300	0.0[e]
Bayleton	92.5[a]
Verdasan	2.5[e]
Du-Ter 50	87.5[b]
Captan	100.0[a]
Plantvax 75	100.0[a]
Dithane M45	97.5[a]
Calixin M	97.5[a]
Delsene M	100.0[a]
Captafol	100.0[a]

Means followed by the same letter are not significantly different (P=0.05).

*Mean of three experiments.

4.2.14 Laboratory screening of various fungal isolates for antagonism towards Sclerotium rolfsii.

As shown in Table 4.16 three types of reactions of S. rolfsii were observed towards other fungi co-inoculated with it on PDA plates. These reactions were:

(a) reaction in which the mycelium of S. rolfsii overgrew the colony of the test fungus (Plate XIII). Twelve out of the 16 fungi tested showed this reaction.

(b) reaction in which the interacting fungal mycelia mutually inhibited each other with none overgrowing the colony of the other. Rather, a narrow zone of inhibition 1 to 3mm wide was formed between the two colonies (Plate XIV). Only two fungi tested, a Trichoderma sp and a strain of Aspergillus flavus showed this reaction.

(c) reaction in which the mycelium of the test organism overgrew the colony of S. rolfsii (Plate XV). This reaction was observed only in the presence of Trichoderma viride and T. harzianum.

It was found that sclerotia produced by S. rolfsii in (a) and (b) above were usually viable and 100%

TABLE 4.16

Reaction between isolate SR-1 of *Sclerotium rolfsii* and some fungi naturally associated with the basal stem rot disease on tomato in parts of northern Nigeria, when co-inoculated on PDA and incubated for 3 days at 30 ± 1°C.

Test Organism	Type of reaction
Sclerotium rolfsii alone (control)	Not applicable
Trichoderma viride	overgrew *S. rolfsii* colony
Trichoderma harzianum	overgrew *S. rolfsii* colony
Trichoderma hamatum	*S. rolfsii* overgrew colony
Trichoderma sp.	No colony overgrowth
Mucor hiemalis	*S. rolfsii* overgrew colony
Rhizopus microsporus	*S. rolfsii* overgrew colony
Fusarium fusarioides	*S. rolfsii* overgrew colony
Aspergillus flavus	*S. rolfsii* overgrew colony
Aspergillus flavus	No colony overgrowth
Fusarium equiseti	*S. rolfsii* overgrew colony
Aspergillus sydowii	*S. rolfsii* overgrew colony
Gliocladium roseum	*S. rolfsii* overgrew colony
Aspergillus niger	*S. rolfsii* overgrew colony
Fusarium solani	*S. rolfsii* overgrew colony
Cunninghmella elegans	*S. rolfsii* overgrew colony
Fusarium sp.	*S. rolfsii* overgrew colony

germination was recorded among these sclerotia when plated on fresh PDA. Sclerotia produced in (c) were invaded by the antagonists and usually did not germinate on fresh media, except a few sclerotia (20%) produced in association with Trichoderma harzianum. It was also observed that Trichoderma viride invaded the colony of S. rolfsii more rapidly than T. harzianum.

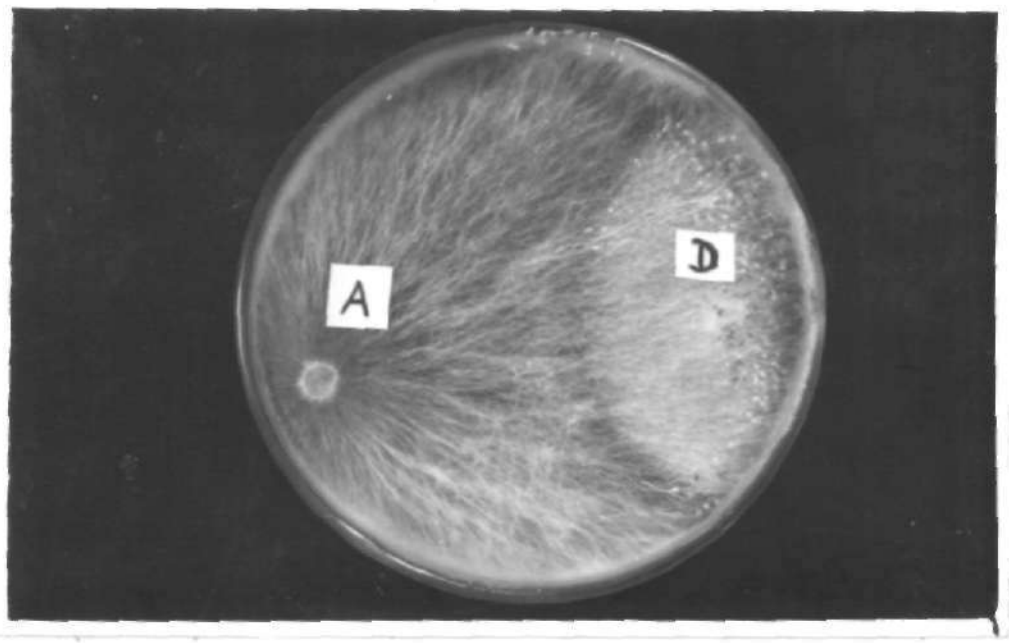


Plate XIII. Mycelium of Sclerotium rolfsii (A) overgrowing colony of Fusarium equiseti (D) on PDA, with sclerotia of A formed mainly over the colony of D.

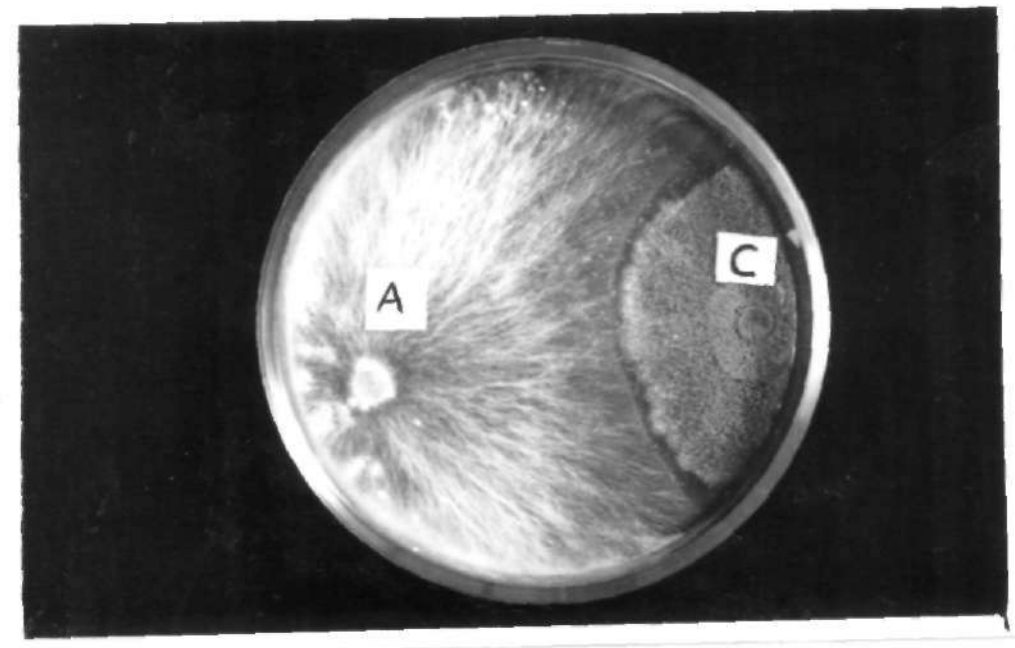


Plate XIV. Mutual inhibition between mycelial colonies of Sclerotium rolfsii (A) and Aspergillus flavus (C) on PDA, with clear narrow zone of inhibition between the two colonies.

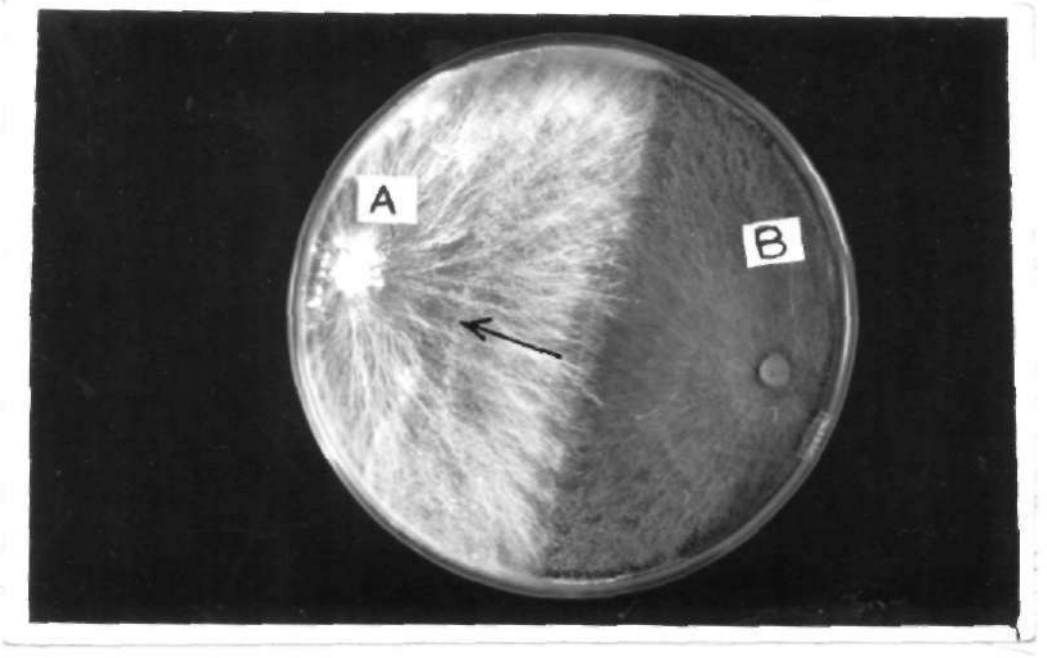


Plate XV. Mycelial colony of Sclerotium rolfsii (A) invaded by mycelium of Trichoderma viride (B) on PDA, indicating the outer margin of the antagonist (arrowed).

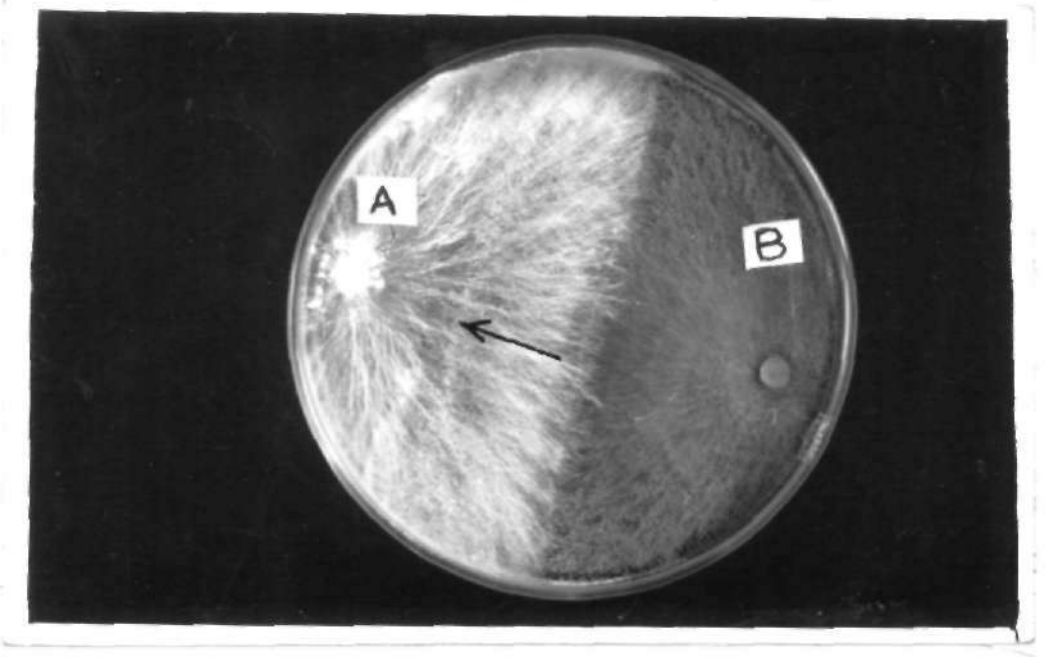


Plate XV. Mycelial colony of Sclerotium rolfsii (A) invaded by mycelium of Trichoderma viride (B) on PDA, indicating the outer margin of the antagonist (arrowed).

CHAPTER 5

5.0 EVALUATION OF VARIOUS FACTORS WHICH INFLUENCE THE DEVELOPMENT OF THE BASAL STEM ROT DISEASE ON TOMATO UNDER GREENHOUSE CONDITIONS.

5.1 Materials and Methods

5.1.1 Sources of seeds used in this study.

Seeds used in this investigation were obtained from the Horticulture and Plant Pathology sections of the Institute for Agricultural Research, Samaru as well as from local markets and commercial stores in Samaru and Zaria.

5.1.2 Tomato cultivar employed in this investigation.

Except where otherwise stated, seedlings of the tomato cultivar 'Roma VF', were used as test crop throughout this study. 'Roma VF' was selected because:

(i) it is a recommended and very popular cultivar among farmers in northern Nigeria.

(ii) it is susceptible to S. rolfsii.

5.1.3 Raising seedlings for greenhouse study.

All tomato seedlings used in this investigation were raised from clean seeds sown in a heat-sterilized mixture of soil and sand (3:1 v/v) in 10 x 33 x 65cm wooden trays

in the greenhouse. When 3 weeks old, seedlings were transplanted, 2 to 5 plants per pot, into sterilized soil in 9 to 19cm diameter plastic pots.

Seedlings of other crops were raised by sowing the seeds or cuttings direct in heat-sterilized soil-sand mixtures (3:1 v/v) in 19cm diameter plastic pots at the rate of 5 seedlings per pot.

5.1.4 Preparation of inocula for greenhouse inoculations.

Three types of inocula of S. rolfsii and other fungi were used for the inoculation of plants in the greenhouse during the course of this investigation. These were: sorghum meal-sand inoculum, sorghum seed inoculum and suspensions of fungal propagules.

5.1.4.1 Sorghum meal-sand inoculum.

The inoculum was prepared by a slight modification of the method proposed by Riker and Riker (1936): Sclerotium rolfsii was grown on autoclaved moist sorghum meal-sand medium (3:1 v/v) in 800ml beakers (the inoculum was difficult to remove when grown in Erlenmeyer flasks). Each beaker was seeded with seven, 5mm discs of young (3 to 4 day-old) cultures of S. rolfsii and then covered with previously autoclaved aluminium foil and incubated for 10 to 12 days at 30 $\pm$ 1°C.

When not immediately needed, the inoculum was removed from the beakers with a flame-sterilized metal spatula, placed in clean trays (or other similar containers) and covered with clean fine cheese cloth before being sun-dried for 2 days (4 hours each day). The inoculum was thereafter stored at room temperature (25° to 28°C) under dry conditions. The inoculum remained infective when used for inoculations after storage for several weeks under these conditions.

5.1.4.2 Sorghum seed inoculum.

Three hundred grammes of sorghum seeds were boiled for 15 minutes in 500ml distilled water containing 5% glucose solution (Avizohar-Hershenzon and Shacked, 1968), and autoclaved in 500ml Erlenmeyer flasks. The flasks were allowed to cool to room temperature (25° - 28°C) before being inoculated with 5mm discs of young (3 to 4 day-old) cultures of S. rolfsii (7 discs per flask). The flasks were incubated at 30 ± 1°C for 5 days. After the incubation period, and when necessary, this inoculum was dried according to the method described earlier above for drying the sorghum meal-sand inoculum of S. rolfsii.

5.1.4.3 Inoculum suspensions.

Mycelial discs, 5mm in diameter, taken from the margin of young cultures of the appropriate fungus were inoculated separately (mycelial part up) in one litre Erlenmeyer flasks, each containing 500ml of potato dextrose broth. The flasks were incubated at 30 ± 1°C for 5 days. The contents of each flask were thereafter placed in a sterile membrane filter inserted in a previously sterilized holder and filtered with the help of an electric vacuum pump (Johnson and Curl, 1972). The fungal mat remaining on each filter was washed in several changes of sterile distilled water to remove traces of stalling material. It was then transferred aseptically into 200ml sterile distilled water containing 5% glucose solution in a sterile Waring blendor and homogenized for 1 minute at low speed.

The concentration of each inoculum suspension was adjusted to 2×10^7 hyphal fragments or conidia/ml using a heamocytometer and a tally counter (Baker, 1967). This was the concentration used for the inoculation of plants in the greenhouse.

5.1.5 Inoculation of plants in the greenhouse.

Except where otherwise stated, the isolate of S. rolfsii used for inoculation of plants throughout this investigation was SR-1 obtained from naturally infected tomato plant in Samaru in September, 1977 (Table 4.1).

Except where otherwise stated, the following procedure was usually adopted for the inoculation of plants with S. rolfsii in the greenhouse: Three grammes of wet or dry sorghum meal-sand inoculum of the pathogen was used per seedling. The inoculum was placed on the surface of soil in pots about 2 to 3mm away from the base of each of 7 to 8 week old seedlings. The inoculum was then covered with heat-sterilized soil to a depth of 5cm. The pots were watered immediately after inoculation and, when necessary, thereafter. Control pots were set up and similarly treated, except that 3g of sterile sorghum meal-sand medium without fungus was placed close to the base of each seedling.

5.1.6 Effect of some factors on disease development in the greenhouse.

These experiments were conducted in the greenhouse using 7 to 8 week old tomato seedlings and at temperatures ranging from 27° to 43°C (mean of 36.3°C). Each experiment

was repeated three times with four replicates in each treatment (a pot containing three seedlings constituting a replicate). These experiments lasted 7 to 14 days.

5.1.7 Effect of age of tomato plant on incidence and severity of the basal stem rot disease in the greenhouse.

Tomato seeds, surface-disinfected by immersion in 0.5% sodium hypochlorite solution for 3 minutes, were sown at weekly intervals for a period of 12 weeks in soil in 19cm plastic pots (10 seeds per pot). When 7 days old, the seedlings were thinned down to 3 per pot. At the end of week 12, a total of 12 seedlings at each age group were inoculated. All seedlings were inoculated on the same day. For each set of inoculated plants, 12 seedlings of the same age were used as non-inoculated controls.

5.1.8 Effect of relative humidity on the development of the basal stem rot disease in the greenhouse.

Two groups of seven-week-old tomato plants consisting of 20 seedlings each (5 seedlings per 12cm diameter pot) were treated as follows: One set of seedlings was inoculated (as described on page 133) with S. rolfsii and then enclosed in clean, moist polyethylene bags 30cm in

diameter in order to achieve high relative humidity inside the bags. A second set was inoculated but not enclosed in polyethylene bags. Two sets, each of 20 non-inoculated control seedlings were also included. One set was enclosed in moist polyethylene bags while the second set was not enclosed.

The relative humidity inside the polyethylene bags and that in the greenhouse was recorded throughout the period of this experiment, using a hair hygrometer with 10.0cm diameter dial. The experiment was conducted at mean greenhouse temperature of 34.2°C. The relative humidity in the atmosphere of the enclosed pots ranged from 80 to 100% while in non-enclosed pots the figure was 50 to 75%.

5.1.9 Effect of mechanical injury on tomato plant on the development of the basal stem rot disease in the greenhouse.

Seven-week-old tomato seedlings separated into four sets were used for this experiment. The first set of seedlings were wounded at the bases of the stems, just above the soil line. Wounds were inflicted as vertical strokes, firmly but lightly made. The wounds were made using a cooled, flame-sterilized inoculating needle. Plants were inoculated immediately after wounding.

The second set of seedlings were left unwounded but inoculated with S. rolfsii. Two sets of control seedlings were set up as follows:

Set I - wounded, non-inoculated plants.

Set II - unwounded, non-inoculated plants.

The experiment was conducted at mean temperature of 34°C.

5.1.10 Effect of artificial defoliation on the development of the basal stem rot disease on tomato in the greenhouse.

All leaves on the test plants, except the youngest two or three leaves surrounding the apical bud, were artificially removed 7 days prior to inoculation. This was done in order to reduce the amount of food available to the plants.

Defoliation was by clipping off the leaves at their bases with a sharp flame-sterilized and cooled pair of scissors, leaving the petiole intact. The resulting scars were immediately sealed with autoclaved and cooled petroleum jelly (vaseline), in order to avoid secondary infection. Three groups of control plants were used. In one group, artificially defoliated plants were left uninoculated and in the other, seedlings with their

leaves intact were inoculated. The third group of control plants consisted of non-defoliated, non-inoculated seedlings. The experiment was conducted at mean greenhouse temperature of 34.3°C, using 8-week-old plants.

5.1.11 Effect of soil type on the development of the basal stem rot disease on tomato in the greenhouse.

Soils used for this test were obtained from different parts of northern Nigeria within the three ecological zones under study (Fig. 1). Collection of soil samples was made in April 1981 (as described on page 75).

All soil samples were sieved separately using 2mm sieves, and 1kg of each sample was amended with 5% sorghum meal (in order to facilitate growth of the pathogen through soil). The amended soil was autoclaved dry and then distributed into 12cm diameter plastic pots. Six grammes of dry sorghum meal-sand inoculum of S. rolfsii was placed 5cm below the soil surface in each pot. The inoculated soils in the pots were kept in the greenhouse for 3 days at mean temperature of 31°C, so that the pathogen could permeate the soils. For each soil sample, there was a control in which soils from the same sample as the infested soil, were inoculated with sterile sorghum meal-sand medium without the fungus.

Tomato seeds were surface-disinfected as described earlier in this study, and then sown in soil in each pot (10 seeds per pot). At the end of the experiment, the soil in pots was turned over and searched for tomato seedlings which germinated but did not emerge. Seedling counts were made 14 days after sowing.

Changes in the pathogenicity of S. rolfsii as influenced by various soil types, were defined in terms of soil conduciveness and determined according to the formula of Henis et al. (1978): $CI = \frac{A - X}{A}$ where,

CI = conduciveness index,

A = number of symptomless seedlings in the non-inoculated control,

X = number of symptomless seedlings in the inoculated treatment, and

A-X = number of plants infected by S. rolfsii.

Values of CI: maximum = 1.00, minium = 0.0

5.1.12 Effect of fungi antagonistic to Sclerotium rolfsii on the development of the basal stem rot disease in the greenhouse.

Two organisms, Trichoderma viride and Aspergillus flavus, which were antagonistic to S. rolfsii in agar plates in this study (Table 4.16) were separately co-inoculated with S. rolsii on tomato seedlings to determine their effect on disease development in the greenhouse.

Ten groups of eight-week-old tomato seedlings were treated as follows:

Groups I and II: Seedlings in each group were first inoculated using a suspension (2×10^7 hyphal fragments/ml) of S. rolfsii. Later, on the same day, each set of seedlings was separately co-inoculated with spore suspensions of T. viride and A. flavus at the same dilution.

Groups III and IV: Seedlings in each group were first inoculated separately with spore suspensions of each antagonist, and three days later, each set of seedlings was co-inoculated with a suspension of S. rolfsii mycelial fragments.

Groups V and VI: Seedlings in each of the two groups were separately inoculated with hyphal suspensions of S. rolfsii, and then also separately co-inoculated, 3 days later, with spore suspensions of each of the antagonists.

Four sets of control plants were employed viz:

(a) plants inoculated with T. viride alone,

(b) plants inoculated with A. flavus alone,

(c) plants inoculated with S. rolfsii alone,

(d) non-inoculated plants.

Inoculation in this experiment was carried out by drenching the soil at the base of each of the inoculated tomato seedlings with approximately 66ml of the appropriate inoculum suspension, and then covering the inoculum with heat-sterilized soil to a depth of 5cm.

5.1.13 Effect of farm yard manures (FYM) on the development of the basal stem rot disease on tomato in the greenhouse.

The droppings of four domestic animals viz: goat, sheep, cow and fowl were used in this experiment. These droppings were collected from the National Animal Production and Research Institute (NAPRI) farm in Shika, near Samaru. The different animal species were kept and fed in separate compartments at NAPRI, so that their droppings did not mix. The droppings were collected using a clean trowel and placed in separate clean polyethylene bags, tied up and stored in the greenhouse until used a few days later. The farm yard manures were not sterilized.

Each of the farm yard manures was incorporated into non-sterile soil at three different levels viz: 2%, 5% and 10% (w/w). Three kilogrammes of soil at each level of the amendment was placed separately in 27cm diameter clay pots. The pots were watered regularly and the contents allowed to equilibrate for three weeks in the greenhouse

at a mean temperature of 34.2°C. At the end of the equilibration period, the contents of the pots were thoroughly mixed and 600g of the amended soil was placed in each of 12cm diameter plastic pots. Three-week-old tomato seedlings were then transplanted into the soils in the plastic pots (three seedlings per pot). The seedlings were inoculated with S. rolfsii four weeks later.

Three sets of control seedlings were set up for each level of the amendments as follows:

(a) non-inoculated seedlings growing in amended soil,

(b) non-inoculated plants growing in non-amended soil, and

(c) inoculated plants growing in non-amended soil.

5.1.14 Effect of crop residues on the pre-emergence damping-off of tomato seedlings caused by Sclerotium rolfsii in the greenhouse.

The leaves, stems (and heads where applicable) of 11 crop plants listed in Table 5.6 were used in this investigation. The plants were collected at the flowering stage and chopped into small pieces, sun-dried and then ground using a mechanical grinder. Each amendment was added to dried non-sterile soil at the ratio of 1:5 (w/w).

Both soil and amendment were thoroughly mixed together, then place in 12cm diameter plastic pots, regularly watered and allowed to equilibrate for 3 weeks in the greenhouse at a mean temperature of 31°C.

At the end of the equilibration period, amended and non-amended soil in pots was inoculated by placing 3g of wet sorghum meal-sand inoculum of S. rolfsii, 5cm below the soil surface. Tomato seeds were sown in each pot (10 seeds per pot), three days after inoculation. Two groups of control plants were set up as follows:

(a) amended non-inoculated soil, and

(b) non-amended, non-inoculated soil.

At the end of the experiment, counts were taken of the number of seedlings which emerged. Soil in pots was turned over and searched for seedlings which germinated but may not have emerged.

5.1.15 Pathogenicity of different isolates of Sclerotium rolfsii on tomato seedlings in the greenhouse.

Thirteen isolates of S. rolfsii from different host plants obtained from various parts of four different ecological zones in Nigeria (Table 4.1) were tested in this experiment. Seven-week-old tomato seedlings (cv Roma VF) growing in 12cm plastic pots (three seedlings per pot),

were inoculated with dry sorghum meal-sand inoculum of each isolate of the pathogen. Control plants received only sterile sorghum meal-sand medium. Greenhouse temperatures during the period of the experiment ranged from 27° to 43°C, (mean of 36.3°C).

5.1.16 Reaction of various tomato cultivars to artificial infection by Sclerotium rolfsii in the greenhouse.

Experiments were conducted in the greenhouse to determine the reaction of different tomato cultivars to artificial infection by isolate SR-1 of S. rolfsii. A total of 35 cultivars were evaluated (Table 5.8).

Eight seedlings of each cultivar (two seedlings in each of 15cm diameter clay pots) were inoculated with wet sorghum meal-sand inoculum of isolate SR-1 of S. rolfsii. The pots were kept in the greenhouse at temperatures ranging from 29° to 38°C (mean of 31.1°C). Non-inoculated controls consisting of eight seedlings were also used for each cultivar. The experiment was carried out three times with each treatment replicated four times.

5.1.17 Investigation on host range of *Sclerotium rolfsii*.

Several reports (Maduewesi, 1975; Ebenebe *et al*., 1977; Erinle, 1977) indicate that many cultivated plants are attacked by *S. rolfsii* in Nigeria. A host range study was therefore carried out in this investigation, partly to verify these reports and partly to establish any new host or non-host plants for the pathogen in this country.

Forty species of flowering plants in 17 taxonomic families were screened in the greenhouse for susceptibility to infection by isolate SR-1 of *S. rolfsii* (Table 5.9). The test plants were raised from seeds, cuttings or tubers in heat-sterilized soil in 19cm diameter plastic pots. Common weeds were dug up with their roots and soil intact and transplanted into pots. Three weeks after planting or transplanting, the seedlings were thinned down to five per pot and seven weeks later, 20 plants of each species were inoculated with *S. rolfsii*. In each case, 10 plants of any given species served as non-inoculated controls. The experiment was repeated three times with four replicates in each treatment and was carried out at greenhouse temperatures which ranged from 29° to 38°C (mean of 31.1°C).

5.1.18 Phytotoxicity of some fungicides to tomato seedlings in the greenhouse.

The 15 fungicides which inhibited growth of S. rolfsii at 125 p.p.m. (a.i.) in laboratory tests (Table 4.12) were screened for phytotoxicity on tomato seedlings in the greenhouse. The concentrations of fungicides used were 50 and 125 p.p.m. (a.i.).

Two hundred millilitres of fungicide suspensions at each concentration were applied as soil drenches to seven week-old tomato seedlings growing in heat-sterilized soil in 12cm plastic pots (two seedlings per pot). Soil to which fungicide suspension was applied was watered immediately afterwards. Soil in control pots was drenched with 200ml of tap water. Observations were recorded daily for 14 days. The experiment was repeated three times with four replicates in each treatment at greenhouse temperatures ranging from 29° to 38°C (mean of 31.1°C).

The phytotoxicity of the various fungicides was assessed using a scale. The scale was based on the observation that while some fungicides only caused leaf burns, others caused stem circumference clearing. The scale used in this work is as shown below:

0 = No visible disorder

1 = 1-15% of leaf area 'burned' or 1-15% of stem circumference cleared.

2 = 16-35% of leaf area 'burned' or 16-35% of stem circumference cleared.

3 = 36-65% of leaf area 'burned' or 36-65% of stem circumference cleared.

4 = 66-100% of leaf area 'burned' or 66-100% of stem circumference cleared.

5 = plant wilted or dead.

Numerical ratings similar to these have been used by Ohh et al. (1978), to assess damage to plants by micro-organisms.

From the above, a phytotoxicity index was determined as follows:

$$\text{Phytotoxicity index} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of affected plants} \times 5} \times 100$$

5, being the highest numerical rating recorded.

5.1.19 Effect of time of fungicide application on the development of the basal stem rot disease on tomato in the greenhouse.

Six fungicides viz: captan, Aldrex T, PCNB, Aatopam-N, Calixin M and captafol which showed no phytotoxicity to tomato seedlings at 125ppm (a.i.) in this study were evaluated in the greenhouse for their

efficacies as protectants and eradicants against the basal stem rot disease. Each of the experiments was repeated three times with four replications in each treatment at greenhouse temperatures ranging from 29° to 38°C (mean 31.1°C). All seedlings were inoculated (as described on page 133) on the same day, using 3g of wet sorghum meal-sand inoculum of isolate SR-1 of S. rolfsii.

Percentage disease reduction in both experiments was calculated according to the formula of Sanders et al. (1978) as follows:

$$1 - \frac{\text{Mean disease rating in treated samples}}{\text{mean disease rating in untreated checks}} \times 100$$

5.1.20 Effect of pre-inoculation treatment with six fungicides on the development of the basal stem rot disease on tomato in the greenhouse.

Seven-week-old tomato seedlings growing in heat-sterilized soil in 12cm diameter plastic pots (two seedlings per pot) were separated into groups of eight seedlings each. A 200 ml suspension of each of the six fungicides (listed above) was applied separately as soil drenches to each group of plants 0,2,4,6,8 and 10 days before inoculation with S. rolfsii. Three sets of control seedlings were set up as follows:

(a) seedlings treated (with fungicides) but not inoculated,

(b) seedlings not treated (with fungicides) but inoculated, and

(c) seedlings not treated (with fungicides) and not inoculated.

Control plants in groups b and c above received 200ml of tap water only per pot.

5.1.2| Effect of post-inoculation treatment with six fungicides on the development of the basal stem rot disease on tomato in the greenhouse.

Seven week-old tomato seedlings grown in heat-sterilized soil in 12cm diameter plastic pots (two seedlings per pot) were separated into groups (eight seedlings per group). Each group of plants received 200ml of suspension of each of six fungicides, applied separately as a soil drench 0,1,2,3, and 4 days after inoculation with S. rolfsii.

Four groups of control seedlings were set up as follows:

(a) seedlings not treated (with fungicides) and not inoculated,

(b) seedlings treated (with fungicides) but not inoculated,

(c) seedlings not treated (with fungicides) but inoculated, and

(d) seedlings not-treated (with fungicides) and not inoculated.

Control plants in groups a, c and d above received 200ml of tap water only per pot.

5.2 Results of evaluation of some factors affecting the development of the basal stem rot disease on tomato under greenhouse conditions.

5.2.1 Effect of age of tomato plant on incidence and severity of the basal stem rot disease in the greenhouse.

Results presented in Table 5.1 show that there were no significant differences in incidence and severity of the basal stem rot disease on plants inoculated with *Sclerotium rolfsii* at ages one-to eight-weeks from sowing. However, disease incidence and severity in nine-to twelve-week-old plants were significantly lower ($P=0.05$) than those in one-to eight-week-old tomato plants.

TABLE 5.1

Effect of plant age on the development of the basal stem rot disease on tomato in the greenhouse at mean temperature of 34°C, 14 days after inoculation.

Age of plants (weeks)	% showing symptoms*	Disease severity index (D.S.I) (%) at following days after inoculation:			
		2	6	10	14
Control(non-inoculated)	0.0	0.0[a]	0.0	0.0	0.0
1	100.0	60.0[f]	100.0	100.0	100.0
2	100.0	53.9[cd]	100.0	100.0	100.0
3	100.0	46.7[e]	100.0	100.0	100.0
4	100.0	47.2[e]	100.0	100.0	100.0
5	100.0	40.0[cd]	100.0	100.0	100.0
6	100.0	40.0[cd]	100.0	100.0	100.0
7	100.0	33.3[bc]	100.0	100.0	100.0
8	100.0	28.3[b]	100.0	100.0	100.0
9	83.3	24.4[b]	80.0	90.0	90.0
10	75.0	27.5[b]	75.0	90.0	90.0
11	75.0	30.0[b]	68.9	73.3	73.3
12	58.3	25.3[b]	62.2	73.3	73.3

* 12 plants were used for each treatment (age)

Means in a column followed by the same letter are not significantly different (P = 0.05).

Seedlings of all ages showed disease symptoms similar to those observed in the field, but seedlings one to six weeks old at the time of inoculation did not show yellowing of the leaves or stems as a disease symptom. All plants one to eight weeks old at the time of inoculation, died six days after inoculation with S. rolfsii. Of those plants inoculated when nine to twelve weeks old, 62 to 80% of them were killed during the same period. Some plants in the later group were still alive 14 days after inoculation.

Disease symptoms or deaths were not observed in non-inoculated control plants.

5.2.2 Effect of relative humidity on the development of the basal stem rot disease on tomato in the greenhouse.

More plants were initially attacked at the higher relative humidity than at the lower relative humidity. But infected plants at the lower relative humidity died more rapidly than those at the higher relative humidty. Thus the disease severity index recorded two and three days after inoculation was significantly higher for plants at the lower relative humidity than for those at the higher relative humidity (Fig. 3). All inoculated plants kept at the lower relative humidity were dead four days after inoculation. In contrast, inoculated plants at the higher relative humidity were killed 7 days following inoculation.

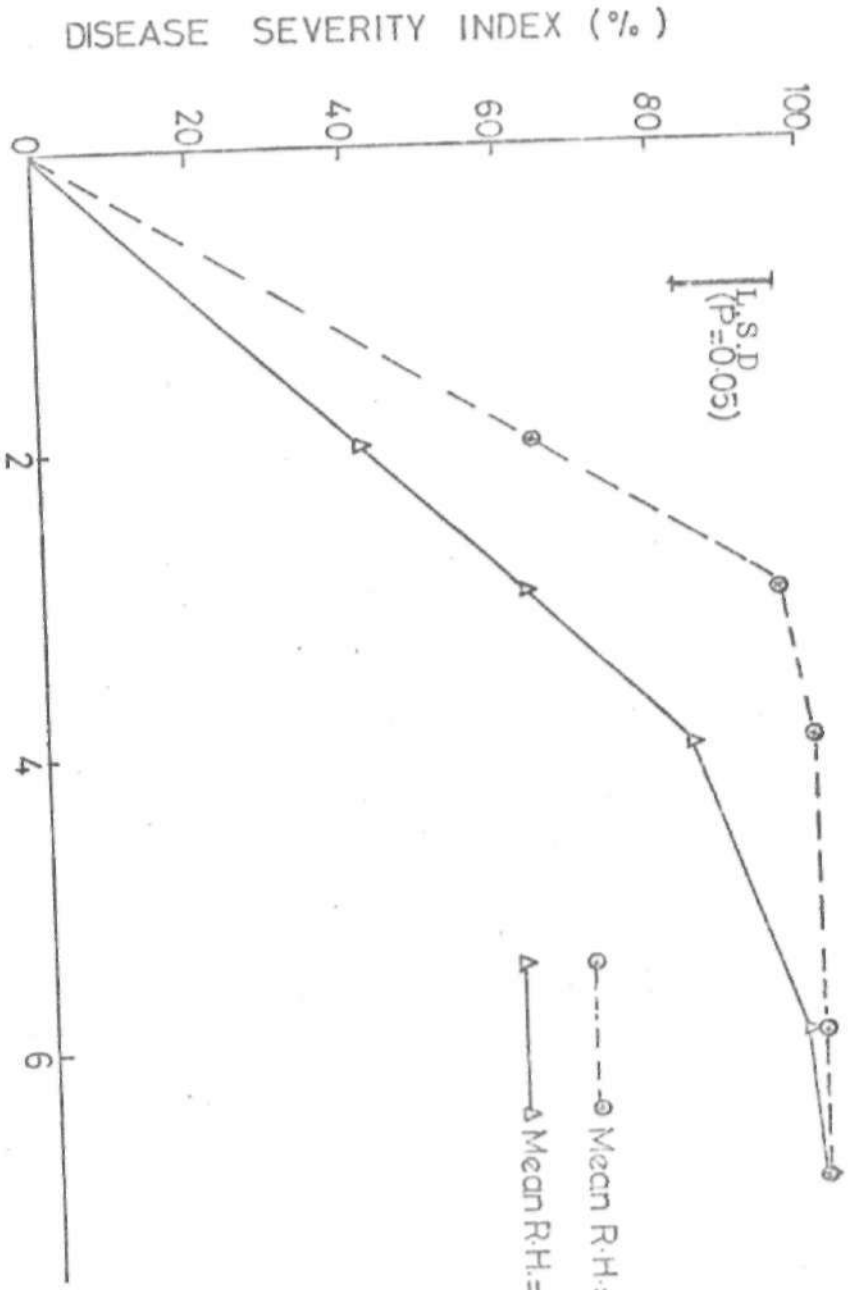


Fig. 3: Effect of relative humidity (R·H) on the development of the basal stem rot disease on tomato in the greenhouse, 7 days after inoculation with isolate SR-1 of sclerotium rolfsii at mean temperature of 34.2°C

Non-inoculated control plants were free of disease symptoms.

Profuse mycelial growth of S. rolfsii was observed on soil surfaces and bases of infected tomato plants in pots kept at the higher relative humidity. It was found that despite this profuse mycelial growth, the formation of sclerotia was delayed for two to three days in such pots. On the contrary, mycelial growth of the fungus on soil surfaces and on the bases of infected tomato stems was scanty in pots kept at the lower relative humidity. Large numbers of sclerotia were readily formed in these pots.

5.2.3 Effect of mechanical injury and defoliation on the development of the basal stem rot disease on tomato in the greenhouse.

Results in Fig. 4 show that disease severity was significantly higher ($P = 0.05$) in wounded tomato seedlings inoculated with S. rolfsii, than in uninjured, inoculated control plants at two days after inoculation. The differences between the two sets of seedlings was most noticeable up to the third day after inoculation. Results were similar for both treatments from the fourth day after inoculation to the seventh day when the trial was terminated and the differences were not significant ($P = 0.05$).

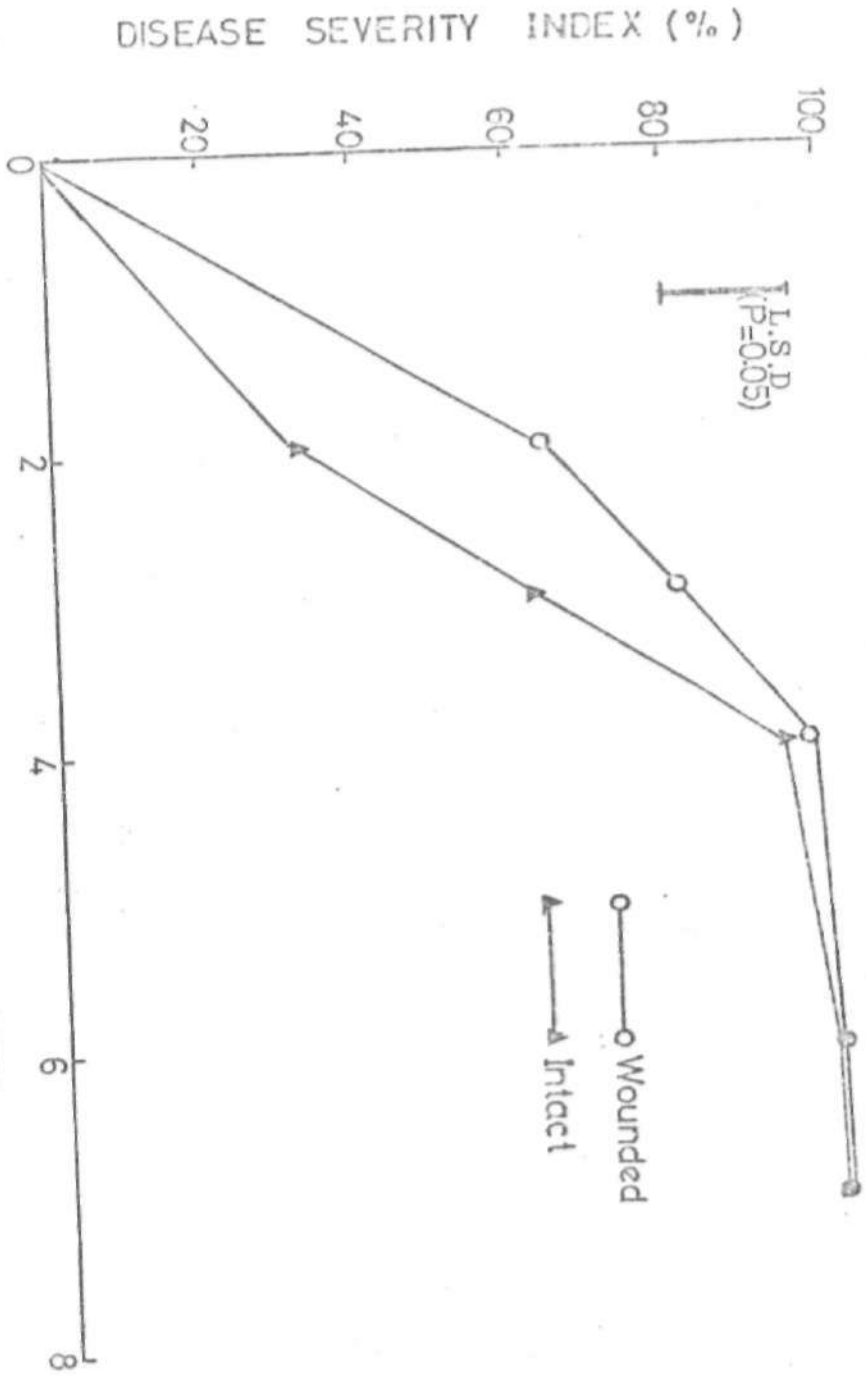


Fig. 4: Effect of mechanical injury on tomato plants on the development of the basal stem not disease in the greenhouse, 7 days after inoculation with isolate SR-1 of Sclerotium rolfsii at mean temperature of 34°C

No deaths or disease symptoms were recorded among seedlings in the wounded and unwounded sets of non-inoculated control plants throughout the period of observation.

Results presented in Fig. 5 show that disease severity in defoliated, inoculated tomato plants was not significantly different (P=0.05) from that in inoculated plants with their leaves intact. Although the differences in these two treatments were not significant, it was observed in the 7 day period after inoculation that tomato seedlings with their leaves intact were killed more rapidly than the defoliated plants. All inoculated plants were killed seven days following inoculation. But control plants neither died nor showed disease symptoms during the same period.

5.2.4 Effect of soil type on the development of the basal stem rot disease on tomato in the greenhouse.

Results presented in Table 5.2 show that the incidence of the basal stem rot disease as expressed by the soil conduciveness index (C.I.) was generally higher in loam and clay-loam soils than in clay soils. They also indicate a negative correlation between organic carbon, nitrogen and phosphorus content of the various soils tested and disease occurrence. A conduciveness index of 0.0 was recorded for the non-inoculated plants.

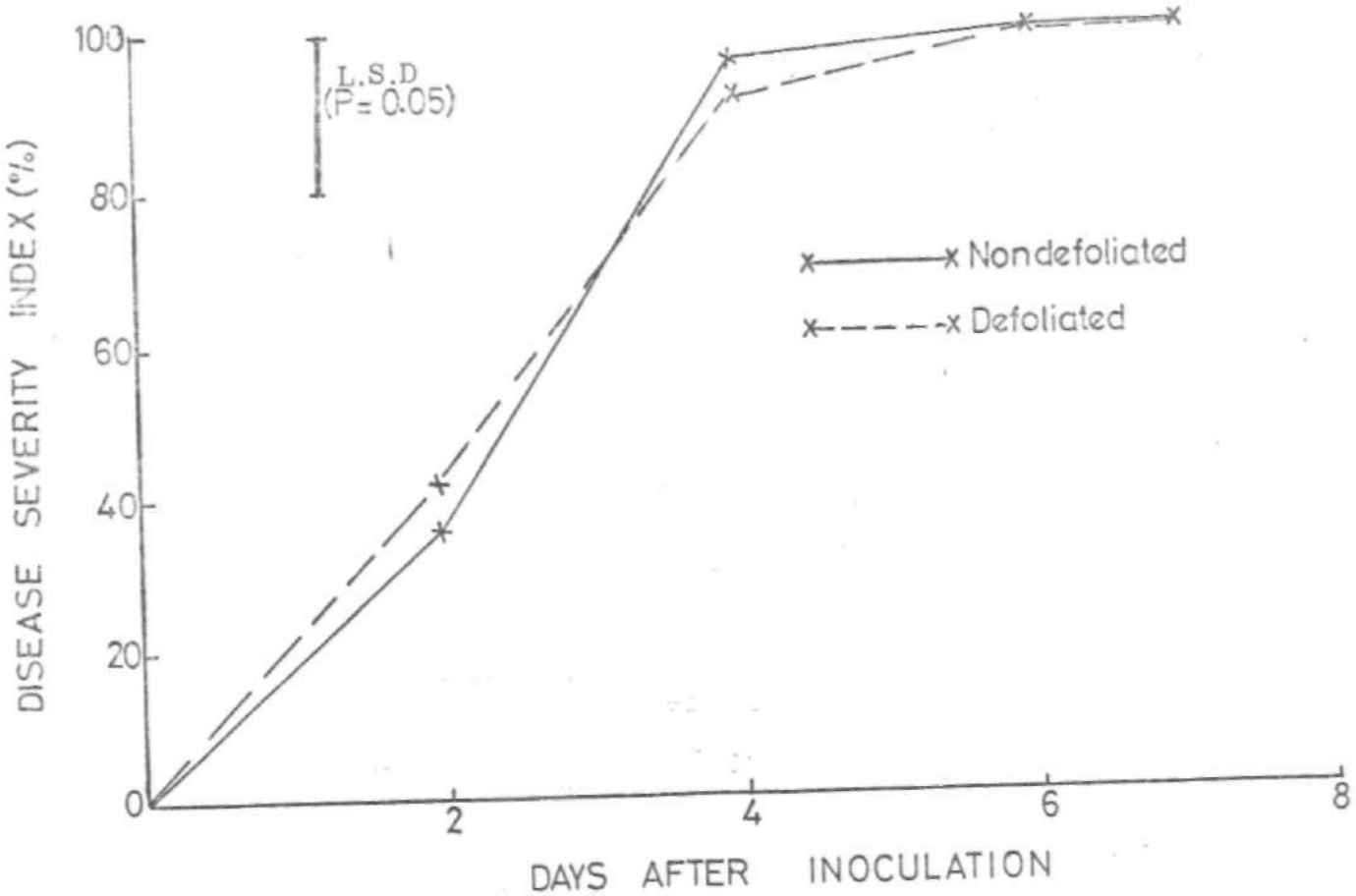


Fig.5: Effect of artificial defoliation of tomato plant, 7days before inoculation with isolate SR–1 of Sclerotium rolfsii, on the development of the basal stem rot disease in the greenhouse at mean temperature of 34·2°C

TABLE 5.2

Effect of soils from various parts of northern Nigeria on the pre-emergence damping-off of tomato seedlings caused by *Sclerotium rolfsii* in the greenhouse, 14 days after sowing at mean temperature of 31°C.

Location	Soil type	No. of symptom-less seed-lings (X)	No. of infected seedlings/seed-lings which did not emerge.	Conducive-ness index (C.I.)
Control (non-inoculated)	Various	30[a]	0	0.0
Kadawa	Clay	9[d]	21	0.70
Riyom	Clay	7[d]	23	0.77
Kafutu	Clay-loam	2[b]	28	0.93
B/Gwari	Clay-loam	5[d]	25	0.83
Gawu	Clay	16[c]	14	0.47
Tumu	Clay	18[c]	12	0.40
Shika	Clay	8[d]	22	0.73
Kontangora	Clay	11[d]	19	0.63
Giwa	Loam	3[b]	27	0.90
Bukuru	Clay-loam	4[b]	26	0.87
Deba Habe	Loam	1[b]	29	0.97
Mokwa	Clay	18[c]	12	0.40
Dangoro	Clay	14[c]	16	0.53
Bauchi	Clay	5[d]	25	0.83
Sokoto	Clay	16[c]	14	0.47
Samaru	Clay-loam	7[d]	23	0.77

Means in a column followed by the same letter are not significantly different (P=0.05).

TABLE 5.3

Physical and Chemical properties of soil from various locations in northern Nigeria.

Location	Physical properties				Chemical properties						
	Soil texture	Sand %	Silt %	Clay %	Organic carbon	Total N(%)	pH (1:1 KCl)	K	Na	Cation exchange capacity	Available P
Kadawa	Clay	12	28	60	0.25	0.0105	5.2	0.217	0.271	14.6	11.20
Riyom	Clay	18	26	56	0.98	0.0245	3.9	0.564	0.065	13.8	0.35
Kafutu	Clay-loam	34	32	34	0.833	0.021	4.8	0.192	0.185	15.9	-
Birnin-Gwari	Clay-loam	12	38	50	0.39	0.0125	5.7	0.385	0.065	13.9	2.45
Gawu	Clay	6	18	76	1.225	0.0245	5.4	0.192	0.043	13.2	10.50
Tumu	Clay	6	22	72	0.294	0.014	5.1	0.115	0.043	11.7	9.80
Shika	Clay	8	30	62	0.49	0.0175	5.2	0.321	0.045	9.9	-
Kontagora	Clay	10	20	70	0.441	0.0175	4.7	0.385	0.076	11.7	8.75
Giwa	Loam	24	50	26	0.833	0.021	3.7	0.410	0.304	19.1	-
Buruku	Clay-loam	18	40	42	0.196	0.007	4.7	0.474	0.076	15.4	1.40
Deba Habe	Loam	42	38	20	0.147	0.007	6.1	0.885	3.586	45.8	3.50
Mokwa	Clay	6	16	78	0.441	0.0175	5.5	0.154	0.054	11.2	12.60
Dangoro	Clay	4	4	92	0.441	0.0175	5.1	0.386	0.054	13.9	8.40
Sokoto	Clay	4	16	80	0.25	0.0105	6.3	0.218	0.054	8.9	28.00
Bauchi	Clay	8	40	52	1.176	0.0283	5.5	0.730	0.076	11.8	18.55
Samaru	Clay-loam	12	42	46	0.294	0.0035	5.5	0.205	0.086	10.4	5.26

The highest conduciveness indices (C.I.) in this study were recorded for soils from Birnin-Gwari, Bauchi, Kafutu, Giwa, Bukuru and Deba Habe. The lowest C.I. recorded were for soils from Tumu, Mokwa and Sokoto. Soils from the remaining localities were intermediate in the C.I. they expressed.

5.2.5 Effect of fungi antagonistic to *Sclerotium rolfsii* on the development of the basal stem rot disease in the greenhouse.

When *Trichoderma viride* was inoculated on tomato seedlings on the same day as, or three days before, inoculation with *S. rolfsii*, the number of plants infected was significantly lower (P=0.05) than the number infected in treatments in which *A. flavus* was used or *T. viride* was co-inoculated three days after *S. rolfsii* (Table 5.4). Results in Table 5.4 also show that both *T. viride* and *A. flavus* were able to reduce disease on tomato plants. But *T. viride* was more efficient than *A. flavus* in reducing disease especially when it was co-inoculated on the same day as, or three days prior, to inoculation with *S. rolfsii*.

TABLE 5.4

Effect of the antagonistic micro-organisms, *Trichoderma viride* and *Aspergillus flavus* when co-inoculated with *Sclerotium rolfsii*, on the development of the basal stem rot disease on tomato in the greenhouse, 14 days after inoculation, at mean temperature of 34°C.

Treatment	Time (days) of application of antagonist relative to time of inoculation with *S. rolfsii*	No. of seedlings showing symptoms out of 16 seedlings per treatment	Disease severity index (D.S.I.) (%)	% Disease reduction
Non-inoculated (control)	Not applicable	0[b]	0.0[b]	Not applicable
S. rolfsii (alone, control)	Not applicable	16[a]	100.0[a]	0.0
T. viride (alone, control)	Not applicable	0[b]	0.0[b]	Not applicable
A. flavus (alone, control)	Not applicable	0[b]	0.0[b]	Not applicable
T. viride + *S. rolfsii*	3 days before *S. rolfsii*	0[b]	0.0[b]	100.0
T. viride + *S. rolfsii*	same day as *S. rolfsii*	0[b]	0.0[b]	100.0
T. viride + *S. rolfsii*	3 days after *S. rolfsii*	16[a]	81.7[a]	18.3
A. flavus + *S. rolfsii*	3 days before *S. rolfsii*	16[a]	58.8[c]	41.2
A. flavus + *S. rolfsii*	same day as *S. rolfsii*	16[a]	53.3[c]	46.7
A. flavus + *S. rolfsii*	3 days after *S. rolfsii*	16[a]	87.0[a]	13.0

Means in a column followed by the same letter are not significantly different (P = 0.05)

All seedlings inoculated with S. rolfsii alone (control) were dead by the time the experiment was terminated. No disease symptoms were recorded in non-inoculated plants or among the plants inoculated either with A. flavus only or T. viride alone.

5.2.6 Effect of farm yard manures (FYM) on the development of the basal stem rot disease on tomato in the greenhouse.

Results in Table 5.5 show that chicken and cow manures applied at the rate of 10%, caused a small disease reduction. The same manures at lower levels of application were ineffective in suppressing disease. Goat and sheep manures were ineffective in reducing disease at all levels of application. However, for each manure, the ability to reduce disease increased with increasing rates of application.

No disease symptoms were detected in non-inoculated control plants. But the disease was less severe in all treatments with farm yard manures than in control plants inoculated with S. rolfsii alone.

TABLE 5.5

Effect of different farm yard manures on the development of the basal stem rot disease on tomato in the greenhouse, 14 days after inoculation at mean temperature of 34.1°C.

Farm Yard Manure (FYM)	Rates of Application (%)		
	2 D.S.I (%)	5 D.S.I (%)	10 D.S.I. (%)
Control (amended soil, non-inoculated)	0	0	0
Control(non-amended Soil, non-inoculated)	0	0	0
Control(non-amended Soil, inoculated)	100.0	100.0	100.0
Chicken	68.3	53.3	31.7
Cow	66.7	41.7	20.0
Goat	76.7	66.7	50.0
Sheep	81.7	75.0	68.3

Means in a column followed by the same letter are not significantly different (P = 0.05)

The pH of the various manures (recorded using pH paper) was as follows:

Chicken manure, pH 6.5 - 7.5
Cow manure, pH 6.5 - 7.5
Goat manure, pH 7.5 - 8.0
Sheep manure, pH 6.5 - 7.5

5.2.7 Effect of crop residues on the pre-emergence damping-off of tomato seedlings caused by *Sclerotium rolfsii* in the greenhouse.

Results in Table 5.6 show that more tomato seedlings emerged without symptoms from treatments containing cereal and grass amendments, than from those containing residues of cowpea, tomato and groundnut. The differences between the two groups of amendments was significant (P=0.05).

The number of plants without symptoms recorded for each of the 11 amendments listed, in Table 5.6, was significantly higher (P = 0.05) than that obtained for non-amended but inoculated controls. No damping-off or disease symptoms were recorded among any of the other two groups of control plants.

TABLE 5.6

Effect of dry crop residues on the pre-emergence damping-off of tomato seedlings in the greenhouse, 14 days after sowing at mean temperature of 31°C.

Dry crop residues	Seedling Emergence		
	No. of symptomless seedlings out of 40 seedlings inoculated per treatment	No. of seedlings damped-off out of 40 seedlings per treatment	No. of seedlings showing symptoms out of 40 seedlings per treatment
Control(amended non-inoculated)	+30[b]	//NA	NA
Control(non-amended, inoculated)	7[a]	20	3[a]
Control(non-amended, non-inoculated)	40[d]	0	0[a]
Cowpea (inoculated)	15[c]	5	20[c]
Tomato (inoculated)	18[c]	12	10[b]
Groundnut (inoculated)	13[c]	11	16[c]
Wheat (inoculated)	35[b]	5	0[a]
Millet (inoculated)	34[b]	6	0[a]
Maize (inoculated)	37[a]	3	0[a]
Sorghum (inoculated)	35[b]	5	0[a]
Hyparrhenia rufa (inoculated)	32[b]	6	2[a]
Hyparrhenia involucrata (inoculated)	35[b]	5	0[a]
Pennisetum typhoides (inoculated)	33[b]	5	2[a]
Pennisetum giganteum (inoculated)	30[b]	7	3[a]

+Mean of all amended non-inoculated treatments in this experiment.

// NA = Not applicable.

Means in a column followed by the same letter are not significantly different (P = 0.05).

5.2.8 Pathogenicity of different isolates of *Sclerotium rolfsii* on tomato seedlings in the greenhouse.

All the 13 isolates of *S. rolfsii* evaluated in this experiment were pathogenic on tomato cultivar, Roma VF.

Some isolates of *S. rolfsii*, however, showed differences in virulence towards the tomato plant. The most virulent of the isolates was isolate SR-8 obtained from cabbage. This isolate of the pathogen killed all the tomato plants with which it was inoculated in two days (Table 5.7), while the other isolates killed all the seedlings in four to six days. The number of seedlings killed by isolate SR-8 on the second day after inoculation was significantly higher ($P = 0.05$) than those killed by each of the other isolates of *S. rolfsii* on the same day. Isolate SR-4 obtained from groundnut was found to be the least virulent. The other 11 isolates of the pathogen were intermediate in virulence towards tomato plants.

No deaths or disease symptoms were recorded for any of the control plants.

TABLE 5.7

Pathogenicity of 13 different isolates of Sclerotium rolfsii on 7-week-old tomato seedlings, in the greenhouse, six days after inoculation.

Isolate of Sclerotium rolfsii and location where obtained	% Seedlings dead		
	Days After Inoculation		
	2	4	6
Control(non-inoculated)	0.0[a]	0.0[b]	0.0
SR-1 (N.G.S.)*	0.0[a]	100.0[a]	100.0
SR-2 (N.G.S.)	0.0[a]	100.0[a]	100.0
SR-3 (N.G.S.)	0.0[a]	100.0[a]	100.0
SR-4 (N.G.S.)	0.0[a]	83.3[c]	100.0
SR-5 (S.G.S.)+	0.0[a]	100.0[a]	100.0
SR-6 (N.G.S.)	0.0[a]	91.7[a]	100.0
SR-7 (S.S.)++	0.0[a]	100.0[a]	100.0
SR-8 (N.G.S.)	100.0[b]	100.0[a]	100.0
SR-9 (S.S.)	0.0[a]	100.0[a]	100.0
SR-10 (N.G.S.)	0.0[a]	91.7[a]	100.0
SR-12 (R.F.)**	0.0[a]	91.7[a]	100.0
SR-13 (N.G.S.)	0.0[a]	97.2[a]	100.0
SR-14 (S.S.)	0.0[a]	100.0[a]	100.0

Means in a column followed by the same letter are not significantly different (P = 0.05).

* N.G.S. = Northern Guinea Savanna

\+ S.G.S. = Southern Guinea Savanna

++ S.S. = Sudan Savanna

++ R.F. = Rain Forest.

5.2.9 Reaction of various tomato cultivars to artificial inoculation with *Sclerotium rolfsii* in the greenhouse.

Table 5.8 shows that all the 35 tomato cultivars screened were susceptible to infection by isolate SR-1 of *S. rolfsii*. The difference in the number of infected seedlings was significant (P = 0.05) in some cultivars but not in others. Based on the disease severity index for each cultivar, three categories of susceptibility of tomato cultivars to *S. rolfsii* were recognised. These were:

(i) Cultivars of low susceptibility to the pathogen (DSI ranging from 0 to 39%) for example Intermech, AT 70/11 and CS 161/69.

(ii) Cultivars of moderate susceptibility (DSI ranging from 40 to 69%) for example M48, Ruby and Campbell 32, and

(iii) Cultivars of high susceptibility (DSI ranging from 70 to 100%) for example Roma VF, Roma VFN and Red River.

No disease symptoms were recorded for non-inoculated control seedlings throughout the duration of the experiment.

TABLE 5.8

Reaction of 35 tomato cultivars to artifical inoculation with *Sclerotium rolfsii*, evaluated 7 days after the inoculation in the Greenhouse at mean temperature of 31.1°C.

Tomato cultivar tested	No. of Seedlings dead out of 8 seedlings inoculated	Disease severity index (D.S.I.) (%)
Control (non-inoculated)	0	0.0
Pavebo Lt.	6	70.6
CS 158/68	4	56.7
Perlita 73	6	71.7
Red River	8	100.0
Rivaldad	5	63.9
Nuova Super Roma	4	50.6
Ventura	7	90.0
Vermillion	5	66.7
Intermech	3	26.7
Lafayette	6	60.0
CS 161/69	3	26.7
Tropic	8	91.1
Heinz 324-1	5	56.7
M 48	4	48.9
Ruby	5	60.6
Super California	7	88.3
Florida MH-1	5	52.2

Table 5.8 (cont'd)

Tomato cultivar tested	No. of seedlings dead out of 8 seedlings inoculated	Disease severity index (D.S.I.) (%)
VF 198 Improved	6	55.0
Castle Mor Improved	8	100.0
Roma VF	8	100.0
Montery	7	74.4
Castle long	8	100.0
Laurano	5	50.6
Luisiana	5	53.6
Castle blend	6	55.6
CS 159/68	7	76.7
AT 70/11	4	26.7
Early Mech	6	57.8
California	4	26.7
Florida Improved	5	46.7
Roma VFN	7	85.0
Campbell 37	8	100.0
Campbell 32	6	56.7
Campbell 34	8	100.0
Sanguinaro 69	8	100.0
L.S.D. (P = 0.05)	2.0	+ND

+ND = Not Determined.

5.2.10 Investigations on the host range of Sclerotium rolfsii.

The results in Table 5.9 established three plants believed to be new host plants for S. rolfsii in Nigeria. These were: Spinach (Amaranthus sp. L.); sorghum (Sorghum vulgare Pers.), and millet (Pennisetum typhoides L.), Except for the weed, 'bahama' grass (Cynodon dactylon L.), all other plants inoculated with S. rolfsii were attacked by the pathogen in this investigation.

Significant differences (P = 0.05) in susceptibility to S. rolfsii were observed between the various families as well as within the families. However, plants in the family Gramineae were the least susceptible to infection by the pathogen. Typical basal stem rot disease symptoms were not observed when plants in this family were inoculated with S. rolfsii. Rather, small, reddish-brown necrotic spots occurred around the collar of infected plants.

Infected plant species with a disease severity index of 70 to 100% were regarded as highly susceptible to infection by S. rolfsii. The most susceptible species observed in this investigation included those in the family Solanaceae, Papilionaceae, and Euphorbiaceae, in which some of the most susceptible plants were tobacco (Nicotiana tabacum L.), tomato (Lycopersicon esculentum Mill.),

TABLE 5.9

Pathogenicity of *Sclerotium rolfsii* to 40 species of crop and non-crop plants, 14 days after inoculation in the greenhouse at mean temperature of 31.1°C.

Common and Scientific name of test plant	Family	Disease severity index (D.S.I.) (%)
Cocoyam (*Xanthosoma sagittifolium* Schott)	Araceae	72.0
Paw-paw (*Carica papaya* L.)	Caricaceae	84.0
Carnation (*Dianthus caryophyllus* L.)	Caryophyllaceae	47.0
Spinach (*Amaranthus* sp.)	Chenopodiaceae	99.0
Sweet potato (*Ipomoea batatas* L.)	Convolvulaceae	79.0
White colosia	Convolvulaceae	100.0
+Cauliflower (*Brassica oleracea* L.)	Crassulaceae	56.7
++Cabbage (*Brassica oleracea* L. cv. Brunswick)	Cruciferae	100.0
Cucumber (*Cucumis sativus* L. cv. 'half long')	Cucurbitaceae	83.3
Water melon (*Citrullus vulgaris* Schrad, cv. Charlestan Gray)	Cucurbitaceae	56.7
Pumpkin (*Cucurbita moschata* Lam. Poir)	Cucurbitaceae	84.0
Cassava (*Manihot esculenta* Granz.)	Euphorbiaceae	100.0

\+ = var. *botrytis*

++ = var. *capitata*

Table 5.9 (cont'd)

Common and Scientific name of test plant	Family	Disease Severity Index (D.S.I.) (%)
Sorghum (Sorghum vulgare pers. cv. 'SL 1499')	Gramineae	33.3
Wheat (Triticum aestivum L.)	Gramineae	21.0
Millet (Pennisetum typhoides L.)	Gramineae	25.0
Rice (Oryza sativa L.)	Gramineae	21.7
Maize (Zea mays L. cv. NCA)	Gramineae	39.0
'Bahama' grass (Cynodon dactylon L.)	Gramineae	0.0
Elephant grass (Pennisetum giganteum L.)	Gramineae	20.0
Onion (Allium cepa L. cv. W 78/B2)	Liliaceae	49.3
Leek (Allium ampeloprasum L.)	Liliaceae	25.7
Cotton (Gossypium hirsutum L. cv. 'Samaru 71')	Malvaceae	58.0
Okra (Hibiscus esculentus L.)	Malvaceae	84.0
Kenaf (Hibiscus cannabinus L.)	Malvaceae	59.0
Roselle (Hibiscus sabdariffa L.)	Malvaceae	46.3
Soyabean (Glycine max (L.) Merr. cv. V40TA)	Papilionaceae	71.7
Bean (Phaseolus vulgaris L.)	Papilionaceae	96.7

Table 5.9 (cont'd)

Common and Scientific name of test plant	Family	Disease severity index (D.S.I.) (%)
Cowpea (Vigna unguiculata L.) Walp. cv. '356 B')	Papilionaceae	100.0
Groundnut (Arachis hypogea L. cv. '187.75')	Papilionaceae	84.3
Pigeon pea (Cajanus cajan (L.) Millsp.	Papilionaceae	81.3
Bambara groundnut (Voandzeia subterranea (L.) D.C.)	Papilionaceae	76.3
Pepper (Capsicum annuum L.)	Solanaceae	87.3
Egg plant (Solanum melongena L. cv. 'Green round type')	Solanaceae	96.7
Potato (Solanum tuberosum L.)	Solanaceae	90.7
Tobacco (Nicotiana tabacum L.)	Solanaceae	100.0
Tomato (Lycopersicon esculentum Mill. cv. Camarillo)	Solanaceae	100.0
Jimson weed (Datura stramonium L.)	Solanaceae	100.0
Jute (Corchorus olitorius L.)	Tiliaceae	58.7
Carrot (Daucus carota L. cv. Nantes improved)	Umbelliferae	100.0
Lemon (Citrus limon (L.) Burm.	Rubtaceae	15.0

Cowpea (Vigna unguiculata L.), groundnut (Arachis hypogea L.); and cassava (Manihot esculenta Granz.) (Table 5.9).

Non-inoculated control plants in all species were free from disease symptoms.

5.2.11 Phytotoxicity of some fungicides to tomato seedlings in the greenhouse.

Table 5.10 shows that seven of the fungicides evaluated were not phytotoxic to tomato seedlings at 50 and 125 p.p.m.(a.i.). These fungicides were: PCNB, Aatopam-N, captan, Dithane M-45, Aldrex T, Calixin M and captafol. The most phytotoxic of the fungicides screened, with phytotoxicity indices of 60 to 100%, were found to be Plantvax 75, Vitavax 75, Vitavax 200 and Vitavax 300. These fungicides were also the only ones which induced the leaf 'burn' effect observed on the leaves of treated tomato plants in this study. Plants showing this disorder were all killed especially at the higher concentration of the fungicides. In leaf 'burn' disorder, the affected parts of the leaves turned gray, and the edges of the leaves gave the appearance of having been burned with fire and rolled upwards.

The least phytotoxic of the fungicides tested were: Ceresan, Delsene M, Du-Ter 50 and Verdasan with phytotoxicity indices of 20 to 60%. These fungicides were also the only

TABLE 5.10

Phytotoxicity of 15 fungicides at concentrations of 50 and 125 ppm (a.i.) to tomato seedlings, 14 days after treatment in the greenhouse at mean temperature of 31.1°C.

a = minor clearing of stem epidermis at soil level,
b = major clearing of stem epidermis at soil level,
c = minor leaf 'burn'
d = major leaf 'burn'

Fungicide and rate (ppm)(a.i.)	Phytotoxicity index (%)	No. of seedlings affected out of 8 seedlings treated	No. of seedlings killed out of 8 seedlings treated	Disorder
Control (no fungicide)	0.0	0	0	Nil
Du-Ter 50				
50	0.0	0	0	Nil
125	40.0	8	0	a
Plantvax 75				
50	40.0	4	4	c
125	60.0	8	8	d
Ceresan				
50	20.0	3	0	a
125	60.0	8	0	b
Delsene M				
50	20.0	1	0	a
125	60.0	1	1	b

Table 5.10 (cont'd)

Fungicide and rate (ppm)(a.i.)	Phytotoxicity index (%)	No. of seedlings affected out of 8 seedlings treated	No. of seedlings killed out of 8 seedlings treated	Disorder
PCNB				
50	0.0	0	0	Nil
125	0.0	0	0	Nil
Verdasan				
50	0.0	0	0	Nil
125	40.0	1	0	a
Aatopam-N				
50	0.0	0	0	Nil
125	0.0	0	0	Nil
Captan				
50	0.0	0	0	Nil
125	0.0	0	0	Nil
Dithane M45				
50	0.0	0	0	Nil
125	0.0	0	0	Nil
Aldrex T				
50	0.0	0	0	Nil
125	0.0	0	0	Nil
Vitavax 75				
50	20.0	3	3	c
125	100.0	8	8	d

Table 5.10 (cont'd)

Fungicide and rate (ppm)(a.i.)	Phytotoxicity index (%)	No. of seedlings affected out of 8 seedlings treated	No. of seedlings killed out of 8 seedlings treated	Disorder
Vitavax 200				
50	40.0	5	3	c
125	100.0	8	8	d
Vitavax 300				
50	60.0	4	4	c
125	100.0	8	8	d
Calixin M				
50	0.0	0	0	Nil
125	0.0	0	0	Nil
Captafol				
50	0.0	0	0	Nil
125	0.0	0	0	Nil
L.S.D. (P=0.05)	+ND	ND	2.97	

+ND = Not Determined.

ones which induced the other disorder observed in this investigation, namely: the clearing of the stems of tomato seedlings near the soil level. In this disorder the epidermal and cortical tissues of the stem were destr yed, leaving a scar (5 to 10mm long) which exposed the woody part of the stem. Tomato plants showing this disorder were not usually killed even at the higher concentration of the fungicides.

Untreated control plants showed no signs of disorder and none was killed during the duration of this experiment.

5.2.12 Effect of pre-inoculation treatment with six fungicides on the development of the basal stem rot disease on tomato in the greenhouse.

Results in Table 5.11 show that all six fungicides evaluated completely prevented the infection of tomato seedlings by S. rolfsii when they were applied to soil zero to four days before inoculation of seedlings with the pathogen. Eight days after application to soil, the effectiveness in disease reduction declined significantly ($P = 0.05$) with four fungicides. These were: Aatopam-N, Aldrex T, Calixin M and captan. At 10 days after application to soil, only PCNB almost completely prevented the infection of tomato seedlings by S. rolfsii. Disease reduction by PCNB

TABLE 5.11

Efficacy of pre-inoculation application of six fungicides 21 days after inoculation at 125 p.p.m. (a.i.) against the basal stem rot disease on tomato seedlings in the greenhouse, at mean temperature of 31.1°C.

Time (days) of inoculation after fungicide application	% Disease reduction with fungicide								
	Controls								
	Non-inoculated, treated with fungicide.	inoculated, not treated with fungicide	non-inoculated, not treated with fungicide	Aatopam-N	Aldrex T	PCNB	Calixin M	Captan	Captafol
0	+NA	0.0	NA	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]
2	NA	0.0	NA	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]
4	NA	0.0	NA	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]
6	NA	0.0	NA	87.5[a]	87.5[a]	100.0[a]	83.4[a]	91.7[a]	75.0[b]
8	NA	0.0	NA	75.0[b]	79.2[b]	100.0[a]	70.8[b]	70.8[b]	50.0[c]
10	NA	0.0	NA	53.3[c]	50.0[c]	95.8[a]	50.0[c]	45.8[c]	20.8[d]

+ NA = Not applicable

Means in a column followed by the same letter are not significantly different (P = 0.05).

at this time was not significantly different (P=0.05) from that at zero or four days after application to soil. The other five fungicides were signficantly (P=0.05) ineffective in reducing disease on tomato seedlings 10 days after application to soil.

All inoculated control plants not treated with fungicides died within the duration of the experiment. No disease symptoms were recorded among all non-inoculated control seedlings during the period of observation.

5.2.13 Effect of post-inoculation treatment with six fungicides on the development of the basal stem rot disease on tomato in the greenhouse.

It was observed in this investigation that 20% of treated tomato seedlings were killed on the second day following inoculation with S. rolfsii, before the application of the fungicides. The rest of the inoculated seedlings died within four days after inoculation.

All inoculated but untreated control plants also died within the four-day period. But non-inoculated control plants (treated and untreated with fungicides) remained free of disease symptoms and no deaths were recorded among them during the period of this study.

CHAPTER 6

6.0 EVALUATION OF SOME FUNGICIDES AND BIOLOGICAL CONTROL AGENTS FOR THE FIELD CONTROL OF THE BASAL STEM ROT DISEASE ON TOMATO.

6.1 Materials and Methods

6.1.1 Raising tomato seedlings for field experiments.

Tomato seedlings for field experiments were raised in nursery beds at the Institue for Agricultural Research Botany Farm. Four or five weeks before sowing, the soil was cultivated and treated with dazomet (Basamid at 40 g/m^2), incorporated to a depth of 20 to 30cm in order to control root-knot nematodes. Single superphosphate was then applied at the rate of 33 g/m^2. Seeds were thinly sown in drills 30cm long and 0.5cm deep. The drills were covered lightly with soil and heavily mulched with grass. The protective mulch material was removed immediately after germination which occurred 5 to 8 days after sowing. The mulch material was carefully re-arranged on either side of the rows of young tomato seedlings in order to reduce the harmful effects of soil splash and erosion during the rains or to conserve soil moisture during the dry season.

The tomato seedlings were then thinned to approximately 20 plants per drill. The seed-beds were watered and weeded when necessary. Seedlings were transplanted to beds in the field when three to four weeks old.

6.1.2 Plots and experimental designs.

Field trials were conducted in the dry seasons of 1978 and 1979 and during the wet season of 1980 to test the effect of some fungicides and antagonistic micro-organisms on the basal stem rot disease on tomato. The experiments were conducted at the Institute for Agricultural Research Farm complex, Samaru (11^{o} 11'N, 7^{o} 38'E, altitude 686m).

Soil in the experimental plots was a clay-loam with a pH of 5.5; 0.294% total C; 0.0035% total N; 0.205 and 0.086 (meq/100g soil) exchangeable K and Na respectively and 5.25 available P.

A completely randomized block design with five replicates (beds) each was used in all experiments. Beds were 1 metre wide and 4.0 metres long, separated by 50cm paths, and covered with grass mulch 5cm high.

6.1.3 Dry season experiment.

The dry season trials were conducted on the same plot in 2 successive years (1978 and 1979). In both years, tomato seeds were sown on November 8. Two weeks before transplanting, the field was cultivated and given a basal dressing of 330 kg/ha of 15:15:15: NPK. Seedlings were transplanted on November 29 and 30 (21 - 22 days after sowing) into slightly sunken beds. The transplants were arranged in two rows of seven seedlings each at a spacing of 60 x 60cm (Quinn, 1974b).

At five weeks after transplanting, side dressings of calcium ammonium nitrate (CAN) at the rate of 160kg/ha were applied on either side of the plants in holes 10 to 15cm from the base of the stems. Plants were inoculated on January 14 (as described below). The beds were irrigated when necessary and at each application, sufficient water was added to saturate the soil.

6.1.4 Wet season experiment.

In the 1980 wet season, field trials were conducted simultaneously in two separate plots two kilometres apart. Except where otherwise stated, the procedure for the wet season experiments was similar to those used in the dry season trials.

In the wet season field trials, seeds were sown on June 5 and seedlings transplanted into raised beds from June 28 to 30. The plants were arranged close to the centre of the beds in double rows 45 x 50cm apart (Quinn, 1980 personal communication), and inoculated on August 14 (as described below).

6.1.5 Application of treatments and plant inoculation in the field.

Three types of treatments were employed in the field experiments. These were:

6.1.5.1 Use of fungicides:

Three protectant fungicides, namely: captan, PCNB and Aldrex T were used. These fungicides had been found to depress the growth of Sclerotium rolfsii in laboratory and greenhouse tests and were non-toxic to tomato plants. Three days before inoculation, the first treatment consisting of 400ml of aqueous suspension of each fungicide, at a concentration of 125 p.p.m. (a.i.), was applied as a soil drench around the base of each plant in beds receiving that particular fungicide. The treatments were repeated after two weeks.

The tomato plants were inoculated when 10 weeks old. To this effect the mulch material was carefully lifted and 3g of dry sorghum seed inoculum of S. rolfsii was scattered by hand around the base of each plant (Gould, 1965). The inoculum was then covered with soil to a depth of about 5cm.

Two groups of control beds were used. These consisted of:

(i) plants inoculated but not treated (with fungicides), and

(ii) plants not inoculated, not treated (with fungicides).

6.1.5.2 Use of micro-organisms.

Two micro-organisms, Aspergillus flavus and Trichoderma viride, which had proved antagonistic to S. rolfsii on agar plates and in greenhouse tests in this study were employed in the present investigation. Inoculum of spore suspensions of each of the antagonists, at a concentration of 2×10^7 propagules/ml, were drenched separately around the bases of plants growing on raised beds. Each plant received 400ml of the appropriate inoculum suspension of the antagonist three days before inoculation with S. rolfsii.

The following controls were used:

(i) plants inoculated with S. rolfsii alone, and

(ii) plants not inoculated, not treated (with micro-organisms).

6.1.5.3 Use of farm yard manures.

Chicken and cow manures were employed. At fairly high levels, these manures had shown some effectiveness in combating the disease in previous greenhouse tests (Table 5.5).

Three weeks after transplanting (when the plants were 6 - 7 weeks old), each farm yard manure was raked (without injuring the plants) into the top 5cm of soil in the appropriate beds at the rate of 1.5 kg/m^2. Plants so treated were then inoculated when 10 weeks old using dry sorghum seed inoculum of the pathogen as already described(on page 185).

The following plots served as controls:

(i) plants inoculated but not treated (with farm yard manures), and

(ii) plants not inoculated, not treated (with farm yard manures).

Disease assessments were made two and four weeks after inoculation (as described on page 42), when the plants were 12 to 14 weeks old.

6.2 Results of the evaluation of some fungicides and biological control agents for the field control of the basal stem rot disease on tomato.

Although Trichoderma viride was slightly more efficient than PCNB, both treatments were the most effective in reducing the basal stem rot disease on dry and wet season tomato crops in Samaru (Tables 6.1 to 6.4). Percentage disease reduction in plots treated with PCNB in the two seasons was significantly higher (P = 0.05) than the results obtained in plots treated with captan or Aldrex T. Disease incidence as well as the total number of infected plants which died were significantly lower (P = 0.05) for PCNB than for captan or Aldrex T during both seasons.

Disease reduction in plots treated with T. viride was significantly higher (P = 0.05) than in plots treated with A. flavus for both seasons, but was not significantly different from that in non-inoculated, non-treated control plots. Treatment with T. viride also gave a significantly lower disease incidence than treatment with A. flavus. Both cow and chicken manures were ineffective in reducing disease in field tomato during the two seasons. The results were similar and were not significantly different from each other or from those in plots treated with S. rolfsii alone. Disease incidence

TABLE 6.1

Effect of some fungicides applied as soil drenches at 125ppm (a.i.), antagonistic micro-organisms and farm yard manures on the development of the basal stem rot disease on tomato in artificially inoculated field plots in Samaru during the 1978/79 dry season.

Disease was evaluated four weeks after inoculation.

Treatment	Disease incidence (%)	No. of plants dead out of 70 plants inoculated	% Disease reduction	Marketable fruit yield in t ha^{-1}
Captan	60.0^{b}	28^{b}	36.9^{c}	14.53
PCNB	25.7^{c}	5^{a}	86.3^{b}	17.47
Aldrex T	65.7^{b}	25^{b}	33.1^{c}	12.60
Aspergillus flavus	100.0^{a}	55^{c}	11.3^{a}	10.34
Trichoderma viride	14.2^{c}	0^{a}	96.6^{b}	18.59
Chicken manure	100.0^{a}	64^{c}	3.7^{a}	11.34
Cow manure	100.0^{a}	59^{c}	5.7^{a}	13.50
Inoculated, non-treated	100.0^{a}	70^{d}	0.0^{a}	9.48
Non-inoculated, non-treated	0.0^{c}	0^{a}	100.0^{b}	18.86

Means in a column followed by the same letter are not significantly different (P = 0.05) according to Duncan's Multiple Range test.

TABLE 6.2

Effect of some fungicides applied as soil drenches at 125 ppm (a.i.), antagonistic micro-organisms and farm yard manures on the development of the basal stem rot disease on tomato in artificially inoculated field plots in Samaru during the 1979/80 dry season.

Disease was evaluated four weeks after inoculation.

Treatment	Disease incidence (%)	No. of plants dead out of 70 plants inoculated	% Disease reduction	Marketable fruit yield in t ha^{-1}
Captan	100.0^{b}	35^{c}	31.4^{a}	14.05
PCNB	54.2^{c}	2^{a}	82.9^{c}	16.90
Aldrex T	100.0^{b}	30^{c}	33.7^{a}	13.54
Aspergillus flavus	100.0^{b}	60^{b}	5.4^{b}	11.63
Trichoderma viride	11.4^{d}	0^{a}	96.9^{d}	17.92
Chicken manure	100.0^{b}	68^{b}	0.6^{b}	10.34
Cow manure	100.0^{b}	65^{b}	1.4^{b}	12.37
Inoculated, non-treated	100.0^{b}	70^{b}	0.0^{b}	9.02
Non-inoculated, non-treated	0.0^{d}	0^{a}	100.0^{d}	17.55

Means in a column followed by the same letter are not significantly different (P = 0.05) according to Duncan's Multiple Range test.

TABLE 6.3

Effect of some fungicides applied as soil drenches at 125 p.p.m. (a.i.), antagonistic micro-organisms and farm yard manures on the development of the basal stem rot disease on tomato in artificially inoculated field plots in Samaru during the 1980 wet season (Site I).

Disease was evaluated four weeks after inoculation.
Total rainfall during crop life was 689.3mm.

Treatment	Disease incidence (%)	No. of plants dead out of 70 plants inoculated	% Disease reduction	Marketable fruit yield in t ha^{-1}
Captan	100.0^{b}	30^{a}	18.6^{d}	12.47
PCNB	28.6^{c}	7^{b}	79.7^{a}	15.54
Aldrex T	100.0^{b}	27^{a}	29.7^{d}	11.33
Aspergillus flavus	100.0^{b}	57^{c}	6.3^{c}	10.25
Trichoderma viride	7.1^{a}	0^{b}	95.7^{b}	16.50
Chicken manure	100.0^{b}	67^{c}	1.1^{c}	10.76
Cow manure	100.0^{b}	62^{c}	3.7^{c}	11.03
Inoculated,non-treated	100.0^{b}	70^{c}	0.0^{c}	8.19
Non-inoculated non-treated	0.0^{a}	0^{b}	100.0^{b}	15.90

Means in a column followed by the same letter are not significantly different (P = 0.05) according to Duncan's Multiple Range test.

TABLE 6.4

Effect of some fungicides applied as soil drenches at 125p.p.m. (a.i.), antagonistic micro-organisms and farm yard manures on the development of the basal stem rot disease on tomato in artificially inoculated field plots in Samaru during the 1980 wet season (Site II).

Disease was evaluated four weeks after inoculation.
Total rainfall during crop life was 689.3mm.

Treatment	Disease incidence (%)	No. of Plants dead out of 70 plants inoculated	% Disease reduction	Marketable fruit yield in t ha^{-1}
Captan	100.0^{b}	38^{c}	15.1^{b}	13.76
PCNB	21.4^{a}	4^{a}	87.6^{c}	16.51
Aldrex T	100.0^{b}	35^{c}	20.8^{b}	11.50
Aspergillus flavus	100.0^{b}	60^{b}	2.9^{a}	10.05
Trichoderma viride	4.3^{a}	0^{a}	97.4^{c}	16.13
Chicken manure	100.0^{b}	65^{b}	2.3^{a}	11.02
Cow manure	100.0^{b}	63^{b}	4.6^{a}	12.24
Inoculated, non-treated	100.0^{b}	60^{b}	0.0^{a}	7.98
Non-inoculated, non-treated	0.0^{a}	0^{a}	100.0^{c}	16.75

Means in a column followed by the same letter are not significantly different (P = 0.05) according to Duncan's Multiple Range test.

for both cow and chicken manures was high (100%) for both the dry and wet season crops. Cow and chicken manures, together with A. flavus, were found to be the least effective treatments for reducing the basal stem rot disease on field tomatoes in this study. All control plants in inoculated, non-treated plots were killed during the period of observation while those in non-inoculated, non-treated plots remained free of disease symptoms and no deaths were recorded among them.

It was observed that incidence of the basal stem rot disease was similar during both dry and wet season field trials. In addition to the basal stem rot disease, wet season tomato crops were attacked by other diseases such as early blight caused by Alternaria solani and septoria leaf spots incited by Septoria lycopersici. Such diseases were not common on dry season crops. But leaf curl and sun scorch affected tomato leaves and fruits during the dry season, respectively.

Data in Tables 6.1 to 6.4 also indicate that marketable fruit yield, in both dry and wet season crops, was highest in plots treated with T. viride, PCNB or in non-inoculated, non-treated control plots. Fruit yield in inoculated, non-treated control plots were the lowest recorded in this investigation.

CHAPTER 7

7.0 DISCUSSION

The present study has shown that the basal stem rot disease caused by Sclerotium rolfsii occurred naturally on several economically important crops in different parts of northern Nigeria. These crops include: tomato (Lycopersicon esculentum Mill.), cabbage (Brassica oleracea L.), onion (Allium cepa L.) and potato (Solanum tuberosum L.) (Table 3.1). This is believed to be the first report of the disease on cabbage in Nigeria. Table 5.9 also shows (apparently, for the first time in Nigeria), that artificially inoculated plants such as spinach (Amaranthus sp. L.), sorghum (Sorghum vulgare Pers.) and millet (Pennisetum typhoides) were infected by the pathogen in the greenhouse.

Some of the records on natural occurrence of the basal stem rot disease (such as on tomato and potato) in this study were made during both the wet and dry seasons. This confirms earlier reports (Quinn, 1974b) that the disease occurs during both seasons in the field.

Results of the field surveys show that the incidence of the basal stem rot disease was always higher on tomato than on any other crop investigated (Table 3.2).

The reason for this frequent attack on tomato plant by S. rolfsii is not known. However, evidence from the present study (Table 4.6) indicates that tomato-stem-extract-agar supported a profuse mycelial growth of the pathogen. The growth in this medium was as good as or even better than the growth in the other plant extract media investigated in this study. Thus, the high incidence of the disease on tomato may be due to the presence in the tomato plant of some principles which favour the growth and development of S. rolfsii more than in other plants. For example, cowpea root exudates have been reported to be unfavourable for the growth of the pathogen due to the absence of certain growth promoting substances in these exudates (Odunfa, 1981).

The incidence of the basal stem rot disease on tomato in the Samaru area was higher in the rainy season than in the dry season, and a close and positive relationship between the amount of rainfall and disease incidence and severity appears possible (Table 3.3).

Observations made during field surveys also indicate that high relative humidity was more favourable to disease incidence on tomato than low relative humidity.

Aycock (1966) also reported that in the tropics, the disease was more prevalent in the wet season when soil moisture levels and relative humidity were high. Severe out-breaks of the basal stem rot disease in other parts of the world have also been associated with the wet season (Rosen and Shaw 1929; Shay, 1953). Similarly, heavy shading and crowding of host plants which tend to increase the moisture content and atmospheric humidity near the soil surface have been reported to favour basal stem rot disease incidence (Avizohar-Hershenzon and Palti, 1962; Aycock, 1966).

During field surveys conducted at Bakori during the dry season by this author, it was found that almost every tomato plant attacked by S. rolfsii had a heavy infestation by the root knot nematode, Meloidogyne spp. Ebenebe et al. (1977) made a similar observation at Mubi (Gongola State of Nigeria). It is possible that, among other factors, wounds inflicted on tomato plants by the root-knot nematode may pre-dispose the plants to attack by S. rolfsii. Observations made in the present investigation (Fig.4), also show that artificially inflicted wounds on tomato seedlings were of initial advantage to the pathogen in the infection of these seedlings.

Sclerotia produced by isolates of S. rolfsii obtained from the northern savanna zones of Nigeria, showed little morphological variation among one another. They had mean diameters of 0.79 to 1.00mm (Table 4.4). One isolate of the pathogen from Ibadan in the rainforest zone of Western Nigeria consistently formed larger sclerotia with diameter ranging from 2.0 to 3.5mm. It is pertinent to note that Maduewesi (1976) and Singh and Allen (1979) reported large sclerotia in isolates of S. rolfsii obtained in the eastern and western rainforest zones of Nigeria respectively. The small size of the sclerotia produced by S. rolfsii in the northern savanna zones of Nigeria may be due to the low organic matter content in the soils in these areas (Table 5.3). This view is supported by the findings of Henis et al. (1965) on sclerotial size and weight in the fungus.

Another variability which was observed in S. rolfsii in this study was the mycelial incompatibility or aversion which occurred between some isolates of the pathogen from different host plants (Table 4.9). This seems to demonstrate the presence of different biochemical strains of S. rolfsii in northern Nigeria. Nakata (1925a) made similar observations on the fungus. It appears that hyphal incompatibility between different isolates of this fungus

may serve as the ultimate means of separating and recognizing different strains which may show close similarities in other characters.

Results of the assay of soils from different fields in Samaru for sclerotia numbers and viability showed that soils from fields BFA and U4 haboured the highest numbers of viable sclerotia (Tables 4.10 and 4.11). These tables also show that tomato was the last crop planted in both fields prior to sampling (c.f. fields D_1, D_2, D_4 and X_2 on Page 74). It appears from these observations that tomato plant supported the build-up of the pathogen in field soils more than day sorghum or groundnut, which were the last crops planted before sampling, in fields which haboured the smallest numbers of viable sclerotia. Crop sequence in farm lands thus appears to have played an important role in determining the incidence of the basal stem rot disease in the fields. Erinle (1980, personal communication) had made similar observations on groundnut in Samaru, while Arene and Okpala (1981) reported that previous monocropping with cocoyam or cassava resulted in a higher incidence of the basal stem rot disease than monocropping with maize or yam.

Laboratory tests in which S. rolfsii was co-inoculated with other fungi which were naturally associated with tomato plants displaying symptoms of the basal stem rot disease showed that the colonies of 75% of these associated microflora were overrun by the mycelium of S. rolfsii (Table 4.16). Only Trichoderma viride and Trichoderma harzianum, representing 12.5% of the test organisms, overran the colony of S. rolfsii in this study. These observations are similar to the reports made by Morton and Stroube (1955), Cooper (1961) and Curl (1974), and may justify the assertion that S. rolfsii may be resistant in natural soil, to antagonism by most of the soil microflora. It is probable that S. rolfsii by its own activities contributes to its own natural control in soil by Trichoderma species. For example, since the acidification of soil is known to favour the growth of Trichoderma species (Weindling, 1932; Adeniji, 1967), it is plausible that S. rolfsii, which normally acidifies the medium in which it is growing (Higgins, 1927), constantly provides a favourable environment for the growth and activity of antagonistic Trichoderma species in soil.

The ability of the pathogen to grow on widely different organic media has also been demonstrated in this investigation (Table 4.6). Thus the ability of S. rolfsii to resist antagonism by other micro-organisms as well as its capacity to grow on different organic media, could enhance its survival potentials in field soils.

As already previously stated, T. viride overgrew the colony of S. rolfsii when co-inoculated on PDA. Results obtained from greenhouse experiments in this study (Table 5.4) also show that no infection was recorded when T. viride was inoculated on tomato plants on the same day as S. rolfsii, or three days prior to co-inoculation with the basal stem rot pathogen. Similar results were obtained in field trials conducted in this study (Table 6.4). Trichoderma viride, in this study, thus appears to have controlled the basal stem rot disease on tomato both in the greenhouse and in the field. Other workers (Mathur and Sarbhoy, 1978; Elad et al., 1980; Tokeshi et al., 1981) also reported excellent control of the basal stem rot disease on sugarbeet, bean and sugarcane respectively, under greenhouse and field conditions using antagonistic Trichoderma species.

Although T. viride has been shown to be highly effective in reducing the basal stem rot disease in this study (Table 6.3), a serious and practical problem remains apparently unsolved. This concerns the method of growth and delivery to field soils of these biological control agents in such a way as to minimize costs. Wells et al. (1972) noted that such a method, in order to be effective, must be cheap, easy to operate and readily standardized. Further research is needed in order to realize these objectives.

Greenhouse tests conducted using soils from various parts of northern Nigeria (Table 5.2), revealed that the incidence of the basal stem rot disease was higher in loam and clay-loam soils than in clay soils. Dubey (1958) and Flados (1958) earlier made similar observations on the preference of S. rolfsii for lighter soil than for heavy clay soil. Both workers attributed this to increased aeration in the lighter soil. Soil texture may thus affect the ability of the pathogen to grow and incite disease in various parts of northern Nigeria, and consequently influence the distribution of the pathogen in these areas. Localities with lighter loam or clay-loam soils may be more conducive to the pathogen than places with heavy clay soils.

Again soil samples from various parts of northern Nigeria have been shown in this investigation to be acidic in nature with pH values of 3.7 to 6.3 (Table 5.3). Results obtained in this study (Table 4.7) suggest that S. rolfsii can grow well over a wide range of pH. These findings show that soils in different localities in nothern Nigeria may be conducive for the growth and pathogenicity of S. rolfsii.

Soil type may be a more important factor than high soil acidity in the development of the basal stem rot disease on tomato, as well as in the geographical distribution of the pathogen. This is because of the inherent capacity of S. rolfsii to produce its own oxalic acid (Avizohar-Hershenzon and Palti, 1962; Bateman and Beer 1965), thus acidifying its own environment and providing the conditions necessary for its own growth and pathogenicity, and therefore becoming less dependent on soil acidity.

Soils from various parts of northern Nigeria were found to be low in nitrogen and organic carbon content (Table 5.3). This may suggest that the occurrence of the basal stem rot disease in these areas is independent of these elements.

All 35 tomato cultivars evaluated in greenhouse tests in this investigation were found to be susceptible to infection by S. rolfsii (Table 5.8). However, the degree of susceptibility varied. Thus cultivars Campbell 34, Roma VF and Red River were among the most susceptible, while cultivars AT 70/11, Intermech and CS 161/69 were among the least susceptible to attack by the pathogen. The number of cultivars evaluated in this study may be small, but the results are in line with the conclusions of Weber and Ramsey (1927) and Mohr and Watkins (1959) that no variety of the cultivated tomato is resistant to infection by S. rolfsii.

In this study, forty different plant species were artificially inoculated in the greenhouse in order to determine the host range of S. rolfsii. The results (Table 5.9) revealed that all but one plant species, namely 'bahama' grass (Cynodon dactylon L.) were susceptible to infection by the basal stem rot pathogen. These results suggest the existence of a wide host range for S. rolfsii among both dicotyledonous and monocotyledonous plant species. Some of these plants were also included in the host list published by Aycock (1966) and Emechebe (1980). Maduewesi (1975) published a long list of crop plants attacked by S. rolfsii in Nigeria.

His list, however, did not include carnation (Caryophyllaceae), spinach (Chenopodiaceae) and carrot (Umbelliferae), which have been shown in this study to be hosts to S. rolfsii. The pathogen may therefore have a much wider host range in Nigeria and elsewhere than is at present recognised.

Results of in vitro screening of 26 fungicides against S. rolfsii (Tables 4.12 and 4.13) revealed that a good number of them were quite effective in inhibiting the mycelial growth of the pathogen. Some of these fungicides, notably the oxanthiin compound (Vitavax), Du-Ter 50, PCNB, Verdasan, Fernasan D and Ceresan actually practically prevented any mycelial growth at active ingredient concentrations as low as 5 to 50 ppm.

In vivo greenhouse tests revealed that of the fungicides which strongly inhibited the mycelial growth of S. rolfsii on PDA, seven fungicides (PCNB, Aatopam-N, captan, Dithane M45, Aldrex T, Calixin M and captafol) appeared to be non-phytotoxic to young tomato plants at the concentrations inhibiting mycelial growth (Table 5.10).

When however these fungicides found to be non-phytotoxic to tomato plants were used for pre-inoculation treatments on tomato seedlings in the greenhouse, PCNB

was found to be the most effective against the basal stem rot disease. It remained effective against the pathogen ten days after application to soil (Table 5.11). Of the remaining fungicides, four, viz: Aatopam-N, Aldrex T, CalixinM and captan were efficacious against the pathogen 4 - 6 days after application to soil. The effectiveness of captafol against the disease was signficantly (P = 0.05) reduced 6 days after application to soil and before inoculation. In post-inoculation treatments, these fungicides were found to be totally ineffective in combating the basal stem rot disease.

Results of field trials carried out in Samaru during both dry and wet seasons in this study (Tables 6.1 to 6.4), showed that of the three fungicides tested (Aldrex T, captan and PCNB) only PCNB was effective in checking the severity of the basal stem rot disease on tomato; it reduced the disease severity index by over 87% in one trial (Table 6.4). In contrast captan and Aldrex T appeared to be ineffective in combating the basal stem rot disease in the field, reducing it by only 15 - 36% and 20 - 33% respectively (Tables 6.1 to 6.4). This finding confirms reports of the successful use of PCNB for the large scale field control of the disease in tomato (Young, 1960; Averre and Jenkins, 1976)

and in several other crops such as groundnut (Harrison, 1961), bulbous iris (Gould and Russell, 1965; Haas, 1976) and bentgrass golf greens (Punja et al., 1982a).

Although T. viride appeared to be as effective as PCNB in controlling the basal stem rot disease on tomato in the greenhouse and in field trials in this study (Tables 5.4 and 6.4 respectively), PCNB (despite its high cost of application), is likely to be more widely employed in controlling the disease than the biological control agent. This is due to the already highlighted operational difficulties associated with the large scale use of biological control agents. Aspergillus flavus as well as chicken or cow manure did not appreciably reduce the incidence of the basal stem rot disease in this study (Tables 6.1 to 6.4).

CHAPTER 8

8.0 SUMMARY AND CONCLUSIONS

Investigations were carried out on the basal stem rot disease and its incitant Sclerotium rolfsii Sacc.. on tomato; fungicidal, biological and some cultural control methods were also investigated.

(1) Field survey conducted over a period of five years (1976 - 1981) in parts of northern Nigeria, indicated that crops such as tomato (Lycopersicon esculentum Mill.), cabbage (Brassica oleraceae L.) and pumpkin (Cucurbita moschata (Lam.) Poir.) were infected in the field, while others such as onion (Allium cepa L.) bulbs and potato (Solanum tuberosum L.) tubers were attacked in storage. Results of field surveys suggest that tomato plant was frequently attacked by the pathogen in the field.

(2) Artificial inoculation in the greenhouse showed that all 35 tomato cultivars evaluated were susceptible to infection by S. rolfsii. Of the 40 different plant species screened for susceptibility to infection by the pathogen (Table 5.9), only 'bahama' grass (Cynodon dactylon L.) appeared to be resistant to infection by S. rolfsii.

(3) Results of field surveys and artificial inoculations in the greenhouse appear to have shown for the first time in Nigeria that cabbage (B. oleraceae L.), spinach (Amaranthus sp. L.), sorghum (Sorghum vulgare Pers.) and millet (Pennisetum typhoides L.) are attacked by the basal stem rot disease.

(4) Aspects of the physiology of S. rolfsii were studied. Observations made on time of initiation of sclerotia and their size showed that different isolates of the fungus obtained from various locations in the Savanna zones of northern Nigeria, consistently formed small sclerotia of mean diameter 0.79 to 1.00mm on PDA in this study (Table 4.4). One isolate of the pathogen from Ibadan in the rain forest zone of western Nigeria, yielded large sclerotia with mean diameter of 2.5mm on PDA. The time of initiation of sclerotia varied widely in different isolates of the fungus. This ranged from two to five days in some isolates (SR-1, SR-2 and SR-5) and seven to twelve days in other isolates (SR-3, SR-7 and SR-12). Mycelial growth and sclerotia formation occurred in the fungus over a wide pH range (pH 2 to pH 14), and the time of initiation of sclerotia appeared not to be affected by the pH of the PDA.

(5) The numbers and viability of sclerotia of S. rolfsii in soils in six fields in Samaru were assessed using the methanol technique and the plant bait method. The results showed that the number of viable sclerotia recorded in soils in which tomato was the last crop planted prior to sampling, was higher than those in fields in which sorghum or groundnut preceeded sampling.

(6) Out of 16 fungi screened for antagonism to S. rolfsii on PDA, only four namely: Trichoderma viride, T. harzianum, Trichoderma sp. and Aspergillus flavus were antagonistic. Trichoderma sp. and A. flavus inhibited S. rolfsii colonies without invading them, while T. viride and T. harzianum invaded the mycelial colonies and sclerotia of the pathogen.

(7) An evaluation of the various factors which influenced the development of the basal stem rot disease on tomato in the greenhouse showed that:

(a) Disease severity index was higher at the lower relative humidity than at the higher relative humidity.

(b) Mechanical injury was found to significantly enhance disease severity.

(c) Incidence of the basal stem rot disease was higher in lighter loam and clay-loam soils than in heavy clay soils.

(d) Trichoderma viride inoculated on the same day as, or three days before, S. rolfsii, was found to be effective in checking the basal stem rot disease on tomato in the greenhouse.

(e) Greenhouse tests in which soil was amended with the residues of several crops (Table 5.6) showed that amendments with cereal and grass residues appeared to reduce basal stem rot disease and favoured the emergence of symptomless tomato seedlings. In contrast, residues of cowpea, groundnut and tomato tended to increase the disease, causing more tomato seedlings to die before emergence.

(8) (a) Results of in vitro screening of 26 fungicides against S. rolfsii (Table 4.12 and 4.13) revealed that a good number of them were quite effective in inhibiting the mycelial growth of the pathogen. Some of these fungicides notably the oxanthiin compounds (Vitavax), Du-Ter 50, PCNB, Verdasan, Fernasan D and Ceresan actually practically prevented any mycelial growth at active ingredient concentrations as low as 5 to 50ppm.

(b) In vivo greenhouse tests revealed that of the fungicides which strongly inhibited the mycelial growth of S. rolfsii on PDA, seven fungicides (PCNB, Aatopam-N, captan, Dithane M45, Aldrex T, Calixin M and captafol) appeared to be non-phytotoxic to young tomato plants at the concentrations inhibiting mycelial growth (Table 5.10).

(9) Six fungicides, viz: PCNB, Aatopam-N, Aldrex T, Calixin M, captan and captafol, were evaluated for disease reduction in pre-and post-inoculation applications in the greenhouse. The results showed that:

(a) PCNB was efficacious in reducing the basal stem rot disease 10 days after application to soil. The efficacy of the other fungicides significantly diminished 4-6 days after application to soil.

(b) The fungicides proved ineffective in checking the disease in all post-inoculation applications.

(10) Field experiments were conducted in Samaru in which the efficacy of the fungicides: Aldrex T, captan and PCNB; the micro-organisms: Trichoderma viride and

Aspergillus flavus as well as animal manures (cow and chicken), were evaluated for ability to control the basal stem rot disease under natural conditions. The results (Tables 6.1 to 6.4) showed that PCNB and T. viride were highly effective in reducing the disease in the field.

In view of the already highlighted operational difficulties associated with the large scale use of T. viride in disease control, PCNB is suggested for field control of the basal stem rot disease on tomato in Samaru.

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APPENDIX A

Composition of agar media employed for the culture of fungal isolates in the present study.

Potato dextrose agar (PDA): Agar, 15.0g; dextrose, 20.0g; distilled water 1000 ml.

Potato dextrose broth: As for PDA less agar.

Czapek-Dox agar: Agar, 15.0g; $NaNO_3$, 3.0g; K_2PHO_4, 1.0g; $MgSO_4.7H_2O$, 0.5g; KCl, 0.5g; $FeSO_4.7H_2O$, 0.01g; Sucrose, 30.0g; distilled water, 1000 ml.

Nutrient agar: Agar, 15.0g; beef extract, 3.0g; peptone; 5.0g; NaCl, 8.0g; distilled water, 1000ml.

Richard's agar: Agar, 15.0g; Sucrose, 50.0g; KNO_3, 10.0g, K_2HPO_4, 5.0g; $MgSO_4.7H_2O$, 2.5g; $FeCl_2$, 0.02g; distilled water, 1000ml.

Malt extract agar: Agar, 25.0g; malt extract (Difco), 20.0g; dextrose, 20.0g; peptone, 1.0g; distilled water, 1000 ml.

Appendix A (Cont'd)

Corn Meal agar: Agar, 17.0g; corn meal 20.0g; distilled water, 1000 ml.

Soil extract agar (Modified): Agar, 15.0g; K_2HPO_4, 0.40g; $(NH_4)_2 HPO_4$, 0.5g; $MgSO_4 . 7H_2O$, 0.05g; $MgCl_2$, 0.1g; $FeCl_3$, 0.01g; $CaCl_2$, 0.1g; pepetone, 1.0g; yeast extract, 1.0; soil extract solution, 250 ml; tap water, 750 ml.

Common Salt agar: Agar, 15.0g; NaCl, 5.0g; distilled water, 1000 ml.

APPENDIX B

Shape and size (mm) of sclerotia of *S. rolfsii* produced on rain-fed tomato plants naturally infected by the basal stem rot disease in Samaru.

Serial No. of sclerotia	Size (mm)	Shape
1	1.00	Spherical
2	1.20	Spherical
3	1.00	Spherical
4	0.90	Spherical
5	0.50	Oblong
6	1.20	Oblong
7	0.70	Oblong
8	0.80	Oblong
9	0.60	Oblong
10	1.00	Oblang
11	1.00	Oblong
12	0.40	Oblong
13	0.30	Flask shaped
14	0.50	Flask shaped
15	0.70	Flask shaped
16	0.80	Oblong
17	0.40	Flask shaped
18	0.60	Spherical

Appendix B (cont'd)

Serial No. of sclerotia	Size (mm)	Shape
19	1.00	Oblong
20	0.50	Oblong
21	0.40	Oblong
22	0.30	Flask shaped
23	0.60	Flask shaped
24	0.70	Flask shaped
25	0.50	Oblong
26	0.70	Oblong
27	0.90	Oblong
28	0.50	Oblong
29	0.60	Oblong
30	0.60	Flask shaped
31	0.70	Flask shaped
32	0.30	Flask shaped
33	1.00	Flask shaped
34	0.80	Spherical
35	0.90	Oblong
36	0.30	Flask shaped
37	0.50	Flask shaped
38	0.90	Flask shaped
39	0.90	Oblong
40	0.90	Oblong

Appendix B (cont'd)

Serial No. of sclerotia	Size (mm)	Shape
41	0.40	Oblong
42	0.40	Oblong
43	0.50	Flask shaped
44	0.40	Flask shaped
45	0.60	Flask shaped
46	0.80	Spherical
47	0.40	Spherical
48	0.50	Spherical
49	0.50	Spherical
50	0.50	Spherical

APPENDIX C

Chemical nature, trade and common names of fungicides evaluated in the laboratory, greenhouse and field for efficiency against *Sclerotium rolfsii*.

Trade Name	Common name and percentage of fungicidal active ingredient	Chemical nature of active ingredient.
Bavistin	Carbendazim (50%)	2-methoxy-carbamoyl-benzimidazole
Zineb	Zineb	Zinc ethylene-1,2-bisdithiocarbamate
Dithane M-45	Mancozeb (80%)	Zinc and manganese ethylene bis dithio-carbamate
Du-Ter 50	Fentin hydroxide (50%)	Triphenyl-tin-hydroxide
Maneb	Maneb	Magnese ethylene-1,2-bisdithiocarbamate
Benlate	Benomyl (50%)	Methyl 1-(butylcarbamoyl) benzimidazole-2-yl carbamate
Calixin	Tridemorph	N-tridecyl-2,6-dimethyl-morpholine
Difolatan 5	Captafol (50%)	N-(1,1,2,2,-tetrachloro-ethane sulphenyl) cyclohex-4-ene-1,2-dicarboximide
Captan	Captan	N-(trichloro methane sulphenyl) Cyclohex-4-ene-1,2-dicarboximide
Quintozene	Terraclor	Pentachloronitrobenzene

Appendix C (cont'd)

Trade Name	Common name and percentage of fungicidal active ingredient	Chemical nature of active ingredient
Plantvax 75w	Oxycarboxin (75%)	5,6-dihydro-2-methyl-N-phenyl-1,4-oxathin-3-carboxamide-4,4-dioxide.
Vitavax 75 w	Carboxin (75%)	2,3-dihydro-6-methyl-5-phenyl carbamoyl-1,4-oxathin.
Vitavax 200	Carboxin (37.5%) + thiram (37.5%)	2,3-dihydro-6-methyl-5-phenylcarbamoyl-1,4-oxathin (bis (dimethyl ethiocarbamoyl) disulphide .
Vitavax 300wp	Carboxin (37.5%) + captan (37.5%)	Carboxin = 2,3-dihydro-6-methyl-5-phenyl carbamoyl-1,4-oxathin. Captan = N-(trichloro methane sulphenyl) cyclohex-4-ene-1,2-dicarboximide).
Ridomil	Metalaxyl	DL-methyl-N-(2,6-dimethyl phenyl)-N-(2-methoxyacetyl) alanineate.
Polyram-Combi	Metiram (80%)	Zinc ethylene thiuram disulphide
Ceresan	Phenyl mercury acetate	Phenyl mercury acetate
Bayleton	Triadimefon	1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl) (butan-2-one.

Appendix C (cont'd)

Trade Name	Common name and percentage of fungicidal active ingredient	Chemical nature of active ingredient
Calixin M	Tridemorph + maneb (47%)	2,6,3-methyl-4-tridecylmorpholine.
Delsene M	Carbendazim (10%) + maneb (64%)	Carbendazim = (methyl benzimidazole-2-yl carbamate). maneb = (manganese ethylene bisdithio-carbamate).
Fernasan D	Thiram (25%) + lindane (20%)	Thiram = (bis (dimethyl ethiocarbamoyl) disulphide). Lindane = 1,2,3,4,5,6-hexachlorocyclohexane.
Tecto	Thiabendazole (60%)	2-(4-thiazolyl)-1H-benzimidazole
Topsin M	Thiophanate-methyl	diethyl-4,4-O-phenyl-enebis-3-thioallophanate
Aldrex T	Thiram (50%) + aldrin (25%)	Thiram = (bis (dimethyl ethiocarbamoyl) disulphide)· aldrin=1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-exb-1,4-endo-5,8-dimethanonaphthalene
Verdasan	Phenyl mercury acetate	Phenyl mercury acetate
Aatopam-N	Thiram (50%) + carbendazim	Thiram = (bis(dimethyl ethiocarbamoyl) disulphide). Carbendazim = methyl benzimidazole-2-yl carbamate.

APPENDIX D

(a) Analysis of variance for effect of different fungicides at concentrations of 125 to 1,000p.p.m. (a.i.) on mycelial growth of Sclerotium rolfsii on PDA.

Source	D.F.	S.S.	M.S.	F.
Main Effects	33	3265.015	98.940	1963.448
Fungicide	30	3210.283	107.009	2123.588
Level	3	54.732	18.244	362.051
2-way interactions	90	211.307	2.348	46.593
Fungicide x level	90	211.307	2.348	46.593
Explained	123	3476.322	28.263	560.871
Residual	248	12.497	0.050	
Total	371	3488.819	9.404	

(b) Analysis of variance for effect of different fungicides at concentrations of 5 to 50 ppm (a.i.) on mycelial growth of S. rolfsii on PDA.

Source	D.F.	S.S.	M.S.	F.
Main effects	19	15601.416	821.127	2150.002
Fungicide	16	14167.715	885.482	2318.507
Level	3	1783.418	594.473	1556.540
2-way interactions	46	1512.519	32.881	86.094
Fungicide x level	46	1512.519	32.881	86.094
Explained	65	17113.935	263.291	689.390
Residual	132	50.413	0.382	
Total	197	17164.348	87.129	

APPENDIX D (cont'd)

(c) Analysis of variance of effect of different media on radial mycelial growth of S. rolfsii.

	Source	D.F.	S.S.	M.S.	F
(i)	Between groups	12	1157827.266	96485.605	64383.351
	Within groups	24	35.966	1.498	
	Total	36	1157863.233		
(ii)	Between groups	12	2709.213	225.767	47.571
	Within groups	24	113.901	4.745	
	Total	36	2823.114		
(iii)	Between groups	12	2931.289	244.274	9.030
	Within groups	24	649.266	27.052	
	Total	36	3580.555		

(d) Analysis of variance for effect of hydrogen ion concentration of PDA on mycelial growth rate in S. rolfsii.

Source	D.F	S.S.	M.S.	F.
Between groups	7	4791.227	684.461	7.966
Within groups	16	1374.720	85.920	
Total	23	6165.947		

(e) Analysis of variance for effect of PDA adjusted to pH 14 on radial mycelial growth of S. rolfsii.

Source	D.F.	S.S.	M.S.	F
Between groups	5	5031.330	1006.266	39.156
Within groups	12	308.383	25.698	
Total	17	5339.717		

Appendix D (cont'd)

(f) Analysis of variance for susceptibility of 35 tomato cultivars to infection by S. rolfsii.

Source	D.F.	S.S.	M.S.	F.
Between groups	34	1383.916	40.703	44.964
Within groups	73	66.083	0.905	
Total	107	1450.000		

(g) Analysis of variance for effect of relative humidity on development of the basal stem rot disease.

Source	D.F.	S.S.	M.S.	F.
Between groups	3	996.000	332.000	442.667
Within groups	8	6.000	0.750	
Total	11	1002.000		

(h) Analysis of variance for effect of sun-drying and surface-disinfection on sclerotial germination in S. rolfsii.

Source	D.F.	S.S.	M.S.	F.
Between groups	4	2097.500	324.400	1311.000
Within groups	10	4.000	0.400	
Total	14	2101.500		

(i) Analysis of variance for effect of wounding tomato plant on disease development.

Source	D.F.	S.S.	M.S.	F.
Between groups	3	920.250	305.750	409.000
Within groups	3	5.000	0.750	
Total	11	925.250		

Appdendix D (cont'd)

(j) Analysis of variance for effect of artificial defoliation of tomato plant on disease development.

Source	D.F.	S.S.	M.S.	F.
Between groups	2	654.000	327.000	981.000
Within groups	6	2.000	0.333	
Total	8	656.000		

(k) Analysis of variance for numbers of viable sclerotia of S. rolfsii in soil.

Source	D.F.	S.S.	M.S.	F.
Between groups	1	1.500	1.500	4.500
Within groups	4	1.333	0.333	
Total	5	2.833		

(l) Analysis of variance for colonization of carrot root and sorghum seed baits by sclerotia of S. rolfsii

(i) Source	D.F.	S.S.	M.S	F.
Between groups	1	1.184	1.184	23.686
Within groups	4	0.200	0.050	
Total	5	1.384		
(ii) Between groups	1	28.166	28.166	12.071
Within groups	4	9.333	2.333	
Total	5	37.500		

Appendix D (cont'd)

(m) Analysis of variance for radial mycelial growth of 14 isolates of S. rolfsii on PDA.

	Source	D.F.	S.S.	M.S.	F.
(i)	Main effects	15	232.051	15.470	4.936
	Isolate	13	201.710	15.516	4.951
	Replication	2	30.341	15.171	4.841
	Explained	15	232.051	15.470	4.936
	Residual	26	81.484	3.134	
	Total	41	313.535	7.647	
(ii)	Main effects	15	441.117	29.408	4.304
	Isolate	13	409.987	31.537	4.615
	Replication	2	31.129	15.565	2.278
	Explained	15	441.117	29.408	4.304
	Residual	26	177.657	6.833	
	Total	41	618.774	15.092	
(iii)	Main effects	15	623.443	41.563	15.295
	Isolate	13	621.920	47.840	17.605
	Replication	2	1.523	0.762	0.280
	Explained	15	623.443	41.563	15.295
	Residual	26	70.653	2.717	
	Total	41	694.096	16.929	