CULTURAL STUDIES ON TWO *CERCOSPORA* SPECIES OF SESAME AND THEIR INTERACTIONS WITH SOME *SESAMUM* GERMPLASM IN KENYA

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

To the memory of my beloved mother

Zipporah Auko Nyanapah

who passed away on 8th July, 1978.

The determination with which she pursued her goals in life has been inspirational to much of my endeavour for success.

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AUDPC-DL - Area under disease progress curve for percent diseased leaves

AUDPC-DF - Area under disease progress curve for percent defoliation

AUDPCs - Area under disease progress curves

CLDA - carrot leaf decoction agar

CLDPDA - carrot leaf decoction potato dextrose agar

CMA - corn meal agar

DRFS - Dryland research field station

FTC - Farmers Training Centre

MEA - malt extract agar

OA - oatmeal agar

PCA - potato carrot agar

PDA - potato dextrose agar

SLDA - sesame leaf decoction agar

SLDOA - sesame leaf decoction oatmeal agar

SLDPDA - sesame leaf decoction potato dextrose agar

SSMA - sesame stem-meal agar

SSMOA - sesame stem-meal oatmeal agar

SSMPDA - sesame stem meal potato dextrose agar

LIST OF APPENDICES

ABSTRACT

Conditions influencing *in vitro* growth and sporulation of *Cercospora sesami* and *C. sesamicola* were investigated to determine appropriate techniques for producing adequate quantities of inocula for use in the creation of epiphytotics of the white and angular leaf spots of sesame during resistance screening programs.

Both fungi produced the largest colonies, and most abundant quantities of conidia on carrot leaf decoction potato dextrose agar, and sesame stem meal agar, respectively. Optimum media quantity for growth of both fungi was 35-ml per 9-cm diameter petri-plate. *C. sesami* sporulated most abundantly in plates holding 15-ml of media, but maximum conidial production by *C. sesamicola* was in those carrying 35-ml. Greatest mycelial and conidial production by the two fungi occurred between pH 6-7. However, prolonged incubation widened the optimum pH range for growth and sporulation of both fungi. Prolonged incubation of *C. sesami* also improved sporulation but only until the 21st day. For *C. sesamicola*, every increase in the duration of incubation enhanced sporulation.

Optimum temperature for culturing both fungi was 25 ^{0} C. Growth of both fungi was stimulated, and depressed by continuous light at all temperatures below and including 30 ^{0} C, and at 35^{0} C, respectively. Sporulation of *C. sesami* was greater under continuous dark and alternating light/dark cycles at temperatures below and including 25^{0} C. At higher temperatures, illumination treatments did not produce significant effects on conidial production. For *C. sesamicola*, the largest number of conidia was observed under continuous light at all the tested temperatures. Although colonies of both fungi, were generally larger when propagated from mycelial fragments than from conidia, the reverse was true for sporulation. A general increase in growth of both fungi occurred following subculturing irrespective of the form of inoculum used to initiate the cultures.

Sporulation of the two fungi also followed a similar pattern when cultures were generated using conidia. For cultures grown using mycelial fragments, subculturing caused a general decline in sporulation of *C. sesami*, but improved that of *C. sesamicola*.

Pathogenicity of *C. sesami* and *C. sesamicola* on cultivated sesame (*Sesamum indicum*) and on three wild species of *Sesamum* were also investigated under glasshouse conditions to provide proof for pathogenicity and establish host range of the two fungi. Within 12 to 28 days following inoculation, both fungi produced symptoms of infection on *S. indicum*, *S. calycinum* and *S. angolense*, but only *C. sesamicola* caused infection on *S. latifolium*. Susceptible sesame plants were also inoculated in the glasshouse to study histopathological relationships of the two fungi. Within 3 to 12-hrs from inoculation, spores of both fungi germinated to give 1 to 6 germtubes per conidium. *C. sesami* did not produce appressoria prior to penetration in contrast to *C. sesamicola*. Penetration of both fungi into sesame leaf tissues was via the stomata and occurred 12 to 60-hrs after inoculation.

White and angular leaf spots were monitored in plots of 16 sesame accessions at Siaya Farmers Training Centre and Kibwezi Dryland Research Field Station to determine relative susceptibility of the accessions to the two diseases. Increases in percentage of diseased leaves and percent defoliation fit the Gompertz model more closely than the logistic model. Rates of disease increase in infected leaves and defoliation as well as areas under disease progress curves (AUDPCs) varied among the sesame accessions studied. Sesame accessions with larger AUDPCs had generally faster rates of disease progress, although this was not always the case. The most susceptible accessions to both diseases were SPS 071 and SIK 134. Accession SPS 013, and accessions SIK 031 and SPS 045 exhibited the least susceptibility to white leaf spot, and angular leaf spot, respectively. These accessions are suggested as future standards for comparing reaction of other genotypes to the respective *Cercospora* leaf diseases of sesame

CHAPTER 1

INTRODUCTION

Consumption of edible vegetable oils in Kenya has risen considerably over the past years, and currently stands at about 5-kg per caput per year. This has been attributed to the constantly increasing population and changing dietary habits in the country. Presently, Kenya's national requirement for vegetable oils stands at about 114,000 metric tonnes, of which only 20 to 30% is produced locally. The massive shortfall in the domestic supply of the commodity is offset through importation, and currently, revenue used for this purpose constitutes the largest proportion of Kenya's food import bills (Ogemma and Ruingu, 1988).

Kenya's dependence on imported vegetable oil can be reduced through increased cultivation of oil crops. However, many of such crops like coconut (*Cocos nucifera*), rape (*Brassica napus*), soybean (*Glycine max*) and sunflower (*Helianthus annus*) give good yields only when they are grown in high potential regions. Unfortunately, most of such regions are already under cultivation of economically more profitable crops such as coffee (*Coffea arabica*), maize (*Zea mays*), and tea (*Camellia sinensis*). Hence, the greatest potential for raising domestic vegetable oil production in Kenya lies in the extension of oil-crop cultivation to the marginal agroecological zones. Among the oil crops grown in Kenya, sesame has the best adaptation to the marginal agroecological zones (Appendix 11). It grows from sea level to about 1500m and can give a crop with as little as 300-mm of rainfall. Sesame was introduced to Kenya by Arabs during the early 1880's in the Lamu-Mombasa region. It later became a major cash crop and export commodity. However, presently sesame is not considered as an important crop in Kenya and its production is confined to the coastal strip and isolated areas in Nyanza and Western provinces. Approximately 5000 ha of land is currently under the crop which is only half the figure realised in 1981 (Gichuki and Gethi, 1988). Yields of up to 2230 kg/ha have been

reported from experimental fields in the country (W'Opindi, 1980), although average yield on farmer's fields is only 80 to 400 kg/ha (W'Opindi, 1981).

Research on sesame is carried out mainly by the Kenya Agricultural Research Institute at Mtwapa in Coast Province. Most of this work involves agronomic and varietal trials (Gichuki and Gethi, 1988). There is also an ongoing research project of the University of Nairobi which aims at developing improved cultivars and agronomic practices.

Sesame seed has high oil content (up to 63%) and low levels of fibre (approximately 5.9%) which makes its oil easier to extract compared to many other oilseed crops. Oil from sesame seed is of high quality (Appendix 12), and can be used in the commercial manufacture of cooking fats, oils, soap, solvents for medicinal formulations, and insecticide synergists. The cake left behind after extracting oil from the seeds can be used as manure and livestock feed. Sesame cake is unique as it can be produced without the need for decortication (Weiss, 1981). Farmers normally consume most of the sesame they produce as roasted seeds, but sometimes the seeds are pounded into a paste and used to make a cake that can be eaten alone or as confectionery (W'Opindi, 1981). Some seed is also used to produce small amounts of vegetable oil that is consumed locally.

Among the production problems that currently limit the production of sesame are damages by various pests and diseases. Pests cause severe damage to the crop and the most notable are webworm (*Antigastra catalaunalis*) and aphids (*Myzus persicae*). A number of diseases have also been observed on sesame in Kenya. These include angular leaf spot (*Cercospora sesamicola* Moh.), white leaf spot (*Cercospora sesami* Zimm.), brown angular leaf spot (*Cylindrosporium sesami* Hansf.), phyllody (MLO), *Phytophthora* blight (*Phytophthora parasitica* (Dastur) var *sesami* Prasad), powdery mildews (*Erysiphe cichoracearum* DC, *Leveillula taurica* (Lev) Trnaud., *Oidium erysyphoides* Fr., *Sphaerothica fuliginea* (Schlecht) Pollacci), stem rots (*Helminthosporium sesami* Miyake, *Phytophthora palmivora* Butl., *Phoma* species, *Rhizoctonia solani* Kuhn.), and wilts (*Fusarium* and *Verticllium*

species) (W'Opindi, 1981; Ayiecho, 1991, personal communication). A preliminary survey conducted on sesame plants growing at the seed multiplication nursery at Siaya Farmers Training Centre (FTC) in June 1991 indicated that white leaf spot and angular leaf spot were the most severe diseases on the crop at the time. White (round) leaf spot of sesame is caused by *Cercospora sesami* Zimm. and was first reported on *S. indicum* L. in Kenya by Gatumbi (1986). Angular leaf spot of sesame is caused by *Cercospora sesamicola* Moh. and has not previously been reported in Kenya. Although sources of resistance to the two *Cercospora* leaf diseases of sesame have been identified in other parts of the world, no studies have been conducted to identify such germplasm in Kenya. In additon, there is limited knowledge about the optimum cultural requirements for production of inocula of *C. sesami* and *C. sesamicola* which can be used in resistance breeding programs. Information is also lacking on the host range of the two fungi and their histopathological relationships on sesame. Such information would be useful in understanding genetic diversity of these fungi and mechanisms of host resistance which are important determinants of the breeding strategies to be employed.

This study was designed to investigate host range, cultural and histopathological characteristics of *C. sesami* and *C. sesamicola*, as well as the reaction of selected germplasm of sesame to leaf spots caused by these fungi. The major objectives of the study were as follows:

1. To identify conditions influencing *in vitro* growth and sporulation of *C. sesami* and *C. sesamicola*.

2. To test the pathogenicity of cultured isolates of *C. sesami* and *C. sesamicola* on *S. indicum*, *S. angolense*, *S. latifolium*, and *S. calycinum*.

3. To study histopathological relationships of *C. sesami* and *C. sesamicola* on susceptible *S. indicum*.

4. To evaluate reaction of 16 *S. indicum* accessions to leaf spots caused by *C. sesami* and *C. sesamicola*. under field conditions.

CHAPTER 2

LITERATURE REVIEW

2.1 History, geographical distribution, and occurrence of *Cercospora* leaf spots

The genus *Cercospora* Fresenius, first described in 1875, has more than 3000 species (Pollack, 1987). Most of these species cause leaf spots in nearly 150 plant families (Chupp, 1953; Vasudeva, 1963; Katsuki, 1965). *Cercospora* species have been documented on many crops including sesame (*Sesamum indicum*) (Nattrass, 1961; Ondieki, 1973; Gatumbi, 1986).

The first description of *Cercospora sesami* Zimm., the causal agent of the white (round) leaf spot of *S. indicum*, dates back to 1904. It has been reported in many countries such as Australia (Anon., 1941), Brazil (Viegas and Torria, 1947), China (Teng and Ou, 1938), Columbia (Barcenas, 1963), Dominican Republic (Ciferri and Gonzalez, 1926), India (Chowdhury, 1944), and Nicaragua (Litzenberger and Stevenson, 1957), Sarawak (Turner *et al.*, 1967). It has also been reported in Somalia (Curzi, 1932), Sri Lanka (Park, 1937), Sudan (Del, 1962), Thailand (Schiller *et al.*, 1981), Uganda (Hansford, 1931), U.S.A (Nusbaum, 1941) and Venezuela (Muller and Chupp, 1942). In Kenya, occurrence of *C. sesami* on *S. indicum* was first reported by Gatumbi (1986), although the fungus had been reported on *S. angolense* much earlier (Nattrass, 1961).

Cercospora sesamicola Moh. which causes the angular leaf spot of *S indicum* was first described in 1958 in India. It has also been reported in Nicaragua (Litzenberger and Stevenson, 1957), Nigeria (Anon., 1965) and Panama (Ferrer, 1960). There is previous documentation of the occurrence of *C. sesamicola* in Kenya. However, symptoms similar to those produced by this fungus were observed on sesame plants growing in experimental plots at

Kibwezi and Siaya (Ayiecho, 1990; personal communication). The symptoms were observed when the crop was about two months old. *Cercospora* leaf spots usually appear in the later stages of crop growth (Hemingway, 1955, 1954; Hilty *et al.*, 1979; Rupe *et al.*, 1982).

2.2 Economic importance of *Cercospora* leaf spots

Varying forms of crop damage have been attributed to attack by *Cercospora* species. These may occur in the field or during storage, and are either of direct or indirect nature (Hemingway, 1954; Gary and Ruppel, 1979; Hilty *et al.*, 1979).

A decline in photosynthetic area due to leaf damage is often the initial effect of *Cercospora* leaf spots. Premature defoliation often follows and in some crops, this may have adverse effects on growth (Hemingway, 1954; Elston *et al.*, 1976). The affected plants may also be rendered more prone to attack by opportunistic organisms (Garry and Ruppel, 1971). In some crops such as maize (*Z. mays* L), stalk rot and breakage may result thereby causing severe crop loss (Hemingway, 1954; Hilty *et al.*, 1979). Reduction in crop quality has also been associated with infection of *Cercospora* species in crops such as sugarbeet (*Beta vulgaris*) (Shanne and Teng, 1992), soybean (Pathan *et al*, 1989), and peanut (*Arachis hypogea*) (Knauft *et al*, 1988). Infection of soybean by *C. kikuchii* has also been attributed to reduction of seed germination (Velicheti *et al.*, 1992).

Yield losses ranging from 50 to 100% have been attributed to *Cercospora* leaf spots in bells of Ireland (*Molucella leavis*) (Njeru, 1988), groundnuts (*Arachis hypogea* L.) (Hemingway, 1954), maize (Roane *et al.*, 1974; Hilty *et al.*, 1979) and sugarbeets (Garry and Ruppel, 1971).

C. sesami causes considerable damage to sesame capsules (Nusbaum, 1941; Muller and Texera, 1941; Chowdhury, 1945; Schiller *et al.*, 1981). Sesame seed yield losses ranging from 20 to 55% have also been attributed to the fungus (Chowdhury, 1945; Barboza *et al.*, 1966; Brar and Ahuja, 1979).

Although *C. sesamicola* also causes significant leaf damage and yield loss on sesame (Ferrer, 1960), there is no documentation on the magnitude of these effects. During the preliminary survey conducted at Siaya FTC, some plants attacked by the fungus exhibited up to 100% leaf necrosis.

2.3 Host range of *Cercospora* species

Although some *Cercospora* species are capable of infecting more than one plant species (Latch and Hanson, 1962; Berger and Hanson, 1963a, Verma *et al.*,1968; Sridharan and Rangaswami, 1968; Pons, 1988; McLean and Roy, 1988), many workers have encountered considerable difficulty in obtaining infection of *Cercospora* species on other plant species different from their natural hosts. Jones (1944) inoculated *Melitotus* species, *Medicago sativa* and *Trifolium pratense* with *Cercospora* cultures from each of these hosts, but obtained infection only on the host from which the culture was derived. Baxter (1956) also inoculated *Medicago sativa M. lupulina, M. hispida, Melitotus officinalis, M. alba* and *T. pratense* with an isolate from *Medicago sativa* but observed infection only on the 3 species of *Medicago*. *C. malayensis* also failed to cause infection on 20 plant species, although it readily infected its natural host (Sridharan and Rangaswani, 1968). There is no previous information on the pathogenicity of *C. sesami* and *C. sesamicola* isolates from *Sesamum indicum* on wild species of *Sesamum*. Although *C. sesami* has been reported on *S. angolense* (Nattrass, 1961), no tests have been conducted to determine whether the *C. sesami* isolated from this host had similar pathogenicity as that from *S. indicum*, or vice versa.

2.4 Histopathological relationships of *Cercospora* species on their respective host plants

At high humidity and temperatures between 16 and 30 ^{0} C, conidia of many *Cercospora* species germinate within 3 to 6 hours to give one or more germtubes on the leaf surface of their hosts such as alfalfa (*Medicago sativa*) (Baxter, 1956) and peanut (Jenkins, 1938; Abdou *et al.*, 1974;

Alderman and Beute, 1986). Penetration of *Cercospora* species into host tissue usually occurs 24 to 48 hours after inoculation and is often via the stomata (Baxter, 1956; Latch and Hanson, 1962; Beckman and Payne, 1981). Generally, no appressoria are formed prior to penetration but in some cases, appressoria formation has been observed on inoculated leaf surface of some plants such as sugarbeet (Solel and Minz, 1971), maize (Thorson and Martison, 1988), and peanut (Melouk and Aboshosha, 1989). There is no previous documentation on the modes of germination or penetration of *C. sesami* nor *C. sesamicola* on sesame leaf surface.

The period between inoculation and symptom expression ranges from 6 to 22 days and is dependent on ambient temperature and humidity conditions; inoculum density, form and virulence; and host's stage of development and inherent susceptibility (Chowdhury, 1944; Latch and Hanson, 1962; Berger and Hanson, 1963b; Gobina *et al.*, 1983; Cooperman and Jenkins, 1986). The lesions produced on the infected tissues are due to necrosis of the invaded plant cells (Chupp, 1953; Solel and Minz, 1971). Important to lesion development is the secretion of a red toxin, cercosporin, by the invading fungal mycelium (Daub, 1982). On illumination, cercosporin is exited to generate free radicals that damage cell membranes thereby causing cell death (Daub, 1987).

Leaf lesions due to *Cercospora* species usually vary in distinctness from faint discoloration to marked necrosis of the infected area (Chupp, 1953). The leaf spots are usually pale to dark-brown in colour with raised margins of darker shade (Ellis, 1976). The lesions may also assume angular, ellipsoidal, round or irregular shape but they are often delimited by leaf veinlets. The size of the lesions usually ranges from 0.5 to 10-mm (Hemingway, 1954; Baxter, 1956; Kingsland, 1963; Deighton, 1974), but the necrotic spots may coalesce to give a blighted leaf appearance under severe infections (Kingsland, 1963; Teri *et al.*, 1980). Premature defoliation of

the infected plants could also occur under heavy infection (Elston *et al.*, 1976). White leaf spot of sesame caused by *C. sesami* is characterized by 0.5 to 5-mm diameter, sub-rotund, white or yellowish-brown centred, dark to purple lesions that are often scattered on both surfaces of the leaf during the early stages of the disease. The lesions later enlarge and may coalesce to give extensive angular or irregular, concentrically zonate necrotic regions of up to 40-mm-diameter. Very severe infection may cause blight or premature defoliation. Under high humidity, bristly growth form on the pale centres of the spots indicating sporulation of the fungus. The petioles, and capsules may also be infected to give lesions of up to 7-mm in size (Muller and Texera, 1941; Chowdhury, 1945; Mazzani, 1966; Schiller *et al.*, 1981).

Angular leaf spot of sesame caused by *C. sesamicola* is characterized by 1 to 8-mm, uniformly angular dark-brown lesions that are often delimited by leaf veinlets on the abaxial surface. On the adaxial leaf surface, the spots are olivaceous brown and fruiting structures of the fungus may appear under high humidity conditions (Mohanty, 1958; Kolte, 1985). Sclerotia may also be produced on both leaf surfaces and these normally appear as dark pycnidia like bodies (Mohanty, 1958; Ferrer, 1960; Rathaiah and Pavgi, 1977).

Sporulation of *Cercospora* species on host surface usually occurs within 21 days following inoculation and is dependent on light, temperature, host susceptibility, and site of infection (Berger and Hanson, 1963b; Nevill, 1981; Gobina *et al.*, 1983; Shew *et al.*, 1989). During sporulation, stromata arise from beneath the epidermis in the substomatal chamber or between the guard cells. As the stromata enlarge, guard cells are forced apart and this usually results into ruptured epidermis. Stromata may also emerge through the stomata and thus remain slightly erumpent on the leaf surface (Berger and Hanson, 1963b; Beckman and Payne, 1981). The stromata later produce conidiophores which are often grouped in fascicles.

2.5 Taxonomy and morphological characteristics of *C. sesami* and *C. sesamicola*

Morphological characteristics of conidia and conidiophores provides the major taxonomic criteria for delimitation of fungal species (Hughes, 1953). *C. sesami* Zimm. and *C. sesamicola* Moh. belong to the family Dematiaceae, order Moniliales of the class Deuteromycetes (Alexopoulos and Mims, 1979).

Conidiophores of *C. sesami* are epiphyllous, unbranched, single or loosely packed fascicles (up to 10), mid-pale olivaceous brown, 0 to 3 septate, apically thickened, nodulose, 40 to 70f by 4 to 5f and each bears 2 to 7 conidia. Conidia are hyaline, straight or slightly curved, subulate, upwardly toothed, 7 to 15 distinctly septate and 90 to 150f by 3 to 4f (Chowdhury, 1944; Vasudeva, 1963; Ellis, 1976). The occurrence of a possible perfect state of the fungus, *Mycosphaerella sesami*, has also been reported (Sivanesan, 1985).

Conidiophores of *C. sesamicola* are produced on dark brown subglobular stromata of 20 to 46f diameter and are normally olivaceous brown, 0 to 2 septate, 15 to 60f by 3 to 43f and arranged in closely packaged fascicles. Conidia are hyaline, cylindric, straight or slightly curved, basically truncate, apically obtuse, subulate, 2 to 7 indistinctly septate and 20 to 120f by 2 to 3f (Mohanty, 1958). The fungus produces abundant sclerotia on both leaf surfaces at crop senescence. These usually appear as dark chestnut-brown, spherical-globose bodies measuring 35 to 70f by 30 to 55f. Some of the sclerotia may transform into spermatogonia and immature perithecia which bear spermatia that measure 35 to 70f by 30 to 85f. This has raised strong possibilities for occurrence of a perfect state of the fungus which is hypothesized to belong to the genus *Mycosphaerella* Johnson (Rathaiah and Pavgi, 1977).

2.6 Control of *Cercospora* leaf spots of sesame

C. sesami is seedborne and can be controlled through a 30 minute, hot water (53 ^{0} C) treatment of seeds (Nusbaum, 1941; Chowdhury, 1945), or fungicides such as 0.15% Carbendazim (Bavistin), Traidimefon (Bayleton) or Thiophamate methyl (Cercobin-M) (Vyas, 1981; Kurozawa *et al.*, 1985). Burning or deep burial of crop residues after threshing or before ploughing can also achieve some control. Although *C. sesamicola* is also seedborne (Ferrer, 1960), it cannot be eliminated from seed through hot water treatment. This is because conidia of the fungus are heat resistant (Rathaiah and Pavgi, 1976) and its mode of perpetuation is mainly through sclerotia in crop debris (Rathaiah and Pavgi, 1973). The greatest potential for controlling the two *Cercospora* leaf spots of sesame lies in the development of disease resistant varieties.

2.7 Techniques used in screening for resistance to *Cercospora* leaf spots

Determination of resistance to foliar diseases in crops can be achieved through various procedures such as assessment of disease incidence and severity in specified plant populations (James, 1974). The study of disease progress using areas-under-disease-progress-curves (Johnson and Beute, 1986) or apparent rates of disease progress (Kranz, 1974 ;Johnson *et al.*, 1986) can also serve the same purpose. Some workers have also screened for resistance to *Cercospora* leaf spots in several pathosystems using various components of plant reaction to disease. These include percent spore germination on inoculated leaf surface, incubation period, lesion size or density per unit of time, rate of lesion expansion or multiplication, and latent period. Other components of resistance to *Cercospora* species are percentage of sporulated lesions after specified periods of incubation, quantity of conidia on sporulated lesions per unit time or lesion area, and time to total necrosis of infected leaf (Melouk

and Banks, 1978; Nevill, 1981; Gobina *et al.*, 1983; Shew *et al.*, 1989). The latter procedures are especially useful for the preliminary evaluation of large amounts of germplasm for resistance in the early stages of the selection program. This is because they are less costly and give faster results in relation to conventional resistance screening techniques.

Resistance of sesame germplasm to *Cercospora* leaf spots has also been reported by several workers (Kushwaha and Kaushal, 1970; Singh *et al.*,1976; Vyas, 1981; Rao and Dhamu, 1983; Kurozawa *et al.*, 1985). However, use of resistance components in the evaluation of sesame germplasm for resistance to white or angular leaf spot has not been documented. This may be due to the previously insufficient knowledge of suitable techniques for *in vitro* production of adequate inocula of *C. sesami* and *C. sesamicola* necessary for creating artificial epiphytotics of the two diseases.

2.8 *In vitro* growth and sporulation of *Cercospora* species

Species of *Cercospora* generally have fastidious requirements for *in vitro* mycelial and conidial production. This makes them grow slowly and sporulate sparsely, or not at all, on most culture media (Cooperman and Jenkins, 1986). The significance of culture media composition to growth and sporulation of *Cercospora* species was first identified in the early 1930's (Nagel and Dietz, 1932; Nagel, 1934). Since then, many workers have investigated the effect of various kinds of media on *in vitro* propagation of members of this genus (Smith, 1971; Beckman and Payne, 1983; Cooperman and Jenkins, 1986). Most *Cercospora* species form small grey mycelial colonies that are devoid of spores on potato dextrose agar (PDA), although a few species can sporulate on this media (Nagel, 1934; Latch and Hanson, 1962; Sobers, 1969). Various media formulations have been developed which favour sporulation of some *Cercospora* species within 3 to 21 days following inoculation. These include host tissue decoction agar (Diachun and Valleau, 1941; El Gholt *et al.*, 1982; Njeru, 1988; Njoya,

1991), host tissue decoction-potato dextrose agar (Abdou and Cooperman, 1974), host-tissue decoction-oatmeal agar (Smith, 1971), oatmeal agar (Abdou, 1966), carrot-leaf decoction agar (Kilpatrick and Johnson, 1956; Latch and Hanson, 1962; Berger and Hanson, 1963a; Roy, 1982; Cooperman and Jenkins, 1986) and V-8 juice agar (Berger and Hanson, 1963a; Ekpo and Esuruoso, 1978; Chen *et al.*, 1979; Cooperman and Jenkins, 1986). Potato carrot agar is also known to favour sporulation of many fungi with meticulous cultural requirements.

Sporulation of *C. sesami* was investigated by Chowdhury (1944) who reported good conidial production on a medium prepared from sesame stems. Kilpatrick and Johnson (1956) also observed moderate sporulation of this fungus on carrot leaf decoction agar; however, assessments made during the study were highly prone to subjective judgement and were carried out after only 5 days of incubation when very little growth of the fungus had occurred. It is possible to improve growth and sporulation of *Cercospora* species on semi-synthetic culture media through prolonged incubation (Njeru, 1988), or addition of a more nutritious component such as potato-dextrose (Nevill and Evans, 1980). There is no previous report of attempts to culture *C. sesamicola* on any media.

In vitro growth of most *Cercospora* species is favoured by slightly acidic-to-neutral pH of culture media (Rangaswami and Chandrasekaran, 1962; Berger and Hanson, 1963a; Verma and Agnihotri, 1972). This is also true for *C. sesami* whose optimal pH for growth is 6.5 (Chowdhury, 1944). There is no previous documentation of the influence of media pH on growth or sporulation of *C. sesamicola*.

Generally, conidial production by *Cercospora* species in culture requires seeding of the media with conidia, since mycelial transfers often yield only sterile vegetative hyphae (Nagel and Dietz, 1932; Nagel, 1934; Ekpo and Esuruoso,1978). However, some workers have managed to obtain spores of *Cercospora* species following inoculation of culture media using mycelial

fragments (Cooperman and Jenkins, 1956), or mycelial discs (Berger and Hanson, 1963a; Verma and Agnihotri, 1972). Subculturing has also been observed to increase sporulation in cultures of some *Cercospora* that were produced from mycelia (Nagel, 1934; Jones, 1958; Calpouzos and Stallknecht, 1965). There is no previous documentation on the preferable forms of inocula for inducing *in vitro* sporulation of *C. sesami* or *C. sesamicola*, nor on effect of subculturing on sporulation of these fungi.

Illumination of incubated culture plates has also been observed to influence growth and sporulation of many *Cercospora* species. In some species, both processes are favoured by continuous illumination (Calpouzos and Stallknecht, 1967) while in others, they are optimal under continuous darkness (Vathakos and Walters, 1979; Cooperman and Jenkins, 1986) or alternating light and darkness (Berger and Hanson, 1963a; Cooperman and Jenkins, 1986). Sporulation of *C. sesami* is reportedly favoured by alternating light and darkness (Chowdhury, 1944). Information on the influence of light on growth or sporulation of *C. sesamicola* was lacking.

Many *Cercospora* species grow and sporulate well within a temperature range of 15 to 30^{0} C (Calpouzos and Stallknecht, 1965; Stavely and Nimmo, 1968; Verma and Agnihotri, 1972). This is also true for *C. sesami* whose growth and sporulation is reportedly optimal at temperatures between 25 and 30^{0} C (Chowdhury, 1944). There was no previous documentation on the effect of temperature on growth or sporulation of *C. sesamicola*.

Although factors influencing *in vitro* growth and sporulation of *Cercospora* species have often been studied in isolation by most workers, interaction of these factors may also influence these process (Calpouzos and Stallknecht, 1965). Thus, more effort should be focused on studying effects of such interactions if optimal combinations of conditions for culturing of these fungi are to be identified.

CHAPTER 3

MATERIALS AND METHODS

3.1 Laboratory Studies

These studies were conducted to culture and identify *C.sesami* and *C. sesamicola* as well as to investigate cultural conditions affecting *in vitro* growth and sporulation of the two fungi.

3.1.1 Isolation and culturing of *C. sesami* and *C. sesamicola*

a) Collection and preservation of diseased specimens

Leaves showing characteristic symptoms of white and angular leaf spots of sesame were collected from sesame plants growing at the University of Nairobi's sesame seed multiplication nursery situated at Siaya Farmers Training Centre (FTC) in Nyanza province. The infected materials were transported to the plant pathology laboratory at Kabete campus, University of Nairobi, and preserved as dried specimens.

(b) Single spore isolation of *C. sesami* and *C. sesamicola*

Monosporic cultures of both fungi were prepared for use in all cultural studies. Single conidia were picked from lesions on infected leaves and seeded onto the surface of water agar using the tip of a sharp sterile inoculating needle. Inoculated plates were incubated on a laboratory bench at room temperature (20 to 25^0 C). Conidial germination on the plates was checked daily using a dissecting microscope and upon germination, agar blocks bearing single germinated conidium were cut out and aseptically seeded onto potato dextrose agar (PDA) plates. The plates were then incubated for 14 days under normal room temperature and lighting conditions (20-24^0 C and 12-hrs daylight).

(c) Maintenance of fungal cultures

Cultures of both fungi were maintained using a modification of the technique described by Boesewinkel (1976). PDA blocks (6-mm^2) bearing actively growing monosporic cultures were aseptically cut out and transferred into sterile universal bottles containing sterile distilled water. The caps were then screwed tight to seal the bottles which were then stored in the refrigerator at 4 to 6 ^{0} C.

3.1.2 Identification of *C. sesami* and *C. sesamicola*

Each fungus was identified using a modification of the slide culture technique described by Riddel (1950). Two sheets of filter paper, a bent glass rod, microscope slide and cover slip were placed in a petri dish in that order and sterilised by steaming without pressure. Sesame stem meal agar medium (sterilized without pressure) was poured into sterile petri dishes to form a layer of about 2-mm depth. Upon solidification, 1-cm^2 agar blocks were cut out using a sharp sterile scalpel and placed on the microscope slide. The centre edges of each agar block were seeded with mycelial fragments using a sterile inoculating needle and the cover slip placed centrally on the block. Petri-plates were covered with lid and incubated as described in 3.1.1. High humidity within the plate was maintained by routine addition of 2 to 3% aqueous solution of glycerine.

When suitable growth and sporulation had occurred, the cover slip was gently lifted using a clean pair of forceps and the agar block discarded. The cover slip was then mounted in a drop of clear lactophenol on a slide and viewed under the light microscope. The colour, size, shape and septation of hyphae, conidiophores and conidia were recorded and compared

with morphological features of the two fungi as described by Ellis (1976) and Mohanty (1958).

3.1.3 Assessment of factors influencing growth and sporulation *C. sesami* and *C. sesamicola*

Similar cultural studies were conducted on *C. sesami* and *C. sesamicola* but the experiments were carried out independently for either fungus.

3.1.3.1 Effect of media composition and quantity

Thirteen types, and three quantities of media were tested. The types of media investigated were carrot leaf decoction agar (CLDA), carrot leaf decoction-potato dextrose agar (CLDPDA), cornmeal agar (CMA), oatmeal agar(OA), potato carrot agar (PCA), potato dextrose agar (PDA), sesame leaf decoction agar (SLDA), sesame leaf decoction-potato dextrose agar (SLDPDA), sesame leaf decoction-oatmeal agar (SLDOA), sesame stem meal agar (SSMA), sesame stem meal potato dextrose agar (SSMPDA), sesame stem meal-oatmeal (SSMOA), and malt extract agar (MEA). Composition of these media is shown in appendix 1. Media quantities tested were 15, 25 and 35-ml per 9-cm diameter petri-plate.

(a) Media and inoculum preparation

All media were sterilized by steaming without pressure for 1-hr at 98^0 C (Kilpatrick and Johnson,1956). The different media quantities (*viz* 15, 25 and 35-ml) were then aseptically pipetted into sterile 9-cm diameter petri-plates.

Inoculum was prepared from 14-day old monosporic cultures that had been grown on PDA as described in 3.1.1. The inoculum comprised of mycelial fragments that were scrapped from the growing colonies using a sterile inoculating needle.

(b) Media inoculation and incubation

Inoculum was transferred to the surface of cool culture media using the tip of a sterile inoculating needle (3 transfers/plate). Inoculated media were placed on the laboratory bench and incubated for 21 days under normal room temperature (20 to 24 ^{0} C) and lighting conditions (12-hr daylight).

(c) Experimental design

The experiment was arranged in a split plot design with media composition, and media quantity as whole-plot, and sub-plot treatments, respectively. Whole-plots were arranged in a completely randomized design with 3 replications. Each subplots consisted of 3 petri-plates.

(d) Measurement of fungal growth

Fungal growth was expressed as radial growth. After each incubation period, radial growth in each petri-plate was measured by the average of two diameters taken at right angles for each colony.

(e) Assessment of conidial production

Conidial production was assessed by determining conidial concentration in suspensions prepared from mycelial discs. The mycelial discs were augured from growing colonies using hollow glass rods. The discs were of 5-mm, and 1-mm diameter in studies with *C. sesami*, and *C. sesamicola*, respectively. The harvested mycelial discs were placed in pyrex tubes containing 3-ml of

50% ethanol-distilled water (nine discs from each subplot per tube). The tubes were agitated for 5 minutes on a Netz motor oscillating shaker (Model IKA-Schutler S 50). Conidial concentration in the resultant suspension was determined using haemacytometer counts. Six counts were made per pyrex tube to give a total of 18 counts per subplot treatment. Each count comprised of the average of values obtained from each of the nine 1-mm^2 squares of the haemacytometer.

(f) Statistical analysis

Averages of colony diameter and number of conidia/ml for each subplot were used for subsequent data analysis. Analysis of variance was carried out at the 1% and 5% probability levels of the F-test. Significant differences in treatment effects identified using Duncan's multiple range test and Least significant difference test at the 1% probability level.

3.1.3.2 Effect of incubation period and media pH

Four incubation periods (viz 7, 14, 21 and 28 days), and six media pH levels (*viz* pH 5, 6, 6.48, 7, 8 and 9) were investigated. Liquid media containing 1% sesame stem meal in distilled water (w/v) was used in the study. The media pH was adjusted using 0.1N HCl or 0.1N NaOH before sterilization. Media with unadjusted pH (pH 6.48) was used to serve as control.

Inoculation was achieved through mycelial fragment transfers (1 transfer per bottle) as described in 3.1.3.1b. Inoculated universal bottles were placed on a mechanical shaker and incubated for the durations specified above under normal room temperature (20-24^0 C) and light conditions (12-hr

daylight).

Split plot experimental design was used with incubation period, and media pH as whole-plot, and sub-plot treatments, respectively. Whole-plots were arranged in a completely randomized design with three replications. Each sub-plot comprised of 3 petri-plates.

After each duration of incubation, mycelial mats were harvested on Whatman's filter paper no. 3 of known weight and dried in the oven for at 60^0 C for 30 to 40-hr. The dried mats were then weighed to determine the amount of fungal growth. Freshly harvested mycelial mats were also placed in pyrex tubes containing 3-ml of 50% ethanol distilled water (w/v). Conidial suspension was then prepared and conidial concentration in the suspension determined as described in 3.1.3.1e.

Averages of mycelial weight and conidial concentration for each sub-plot were then computed and statistical analyses carried out as described in 3.1.3.1f.

3.1.3.3 Effect of temperature and light regimes

Five temperatures (*viz.* 15, 20, 25, 30, and 35^0 C), and three light regimes (*viz.* continuous light, continuous darkness and 12-hr alternating light/dark cycles) were tested.

Mycelial fragments were inoculated onto plates containing 25-ml of solidified sesame stem meal agar medium as described in 3.1.3.1b. Inoculated plates were incubated on shelves located within Gallenkamp

incubators where temperatures were maintained at appropriate level as determined by the temperature treatment (*viz* 15, 20, 25, 30, or 35 ^{0} C). The interior of the incubators were illuminated continuously using two 30W cool-white fluorescent tubes (Phillips FL 8D) mounted 10-cm above the first shelf. Petri-plates for assessing effects of continuous darkness, and alternating light/dark cycles were wrapped using aluminium foil throughout the experiment, and at 12-hr intervals, respectively.

The experiment was arranged in a split plot design with incubation temperature, and lighting regime as whole-plot, and subplot treatments, respectively. Whole-plots were arranged in randomized complete block design with three replications. Blocks represented the position of shelves holding inoculated plates in relation to the source of light within the incubator. Each sub-plot comprised of 3 petri-plates.

Fungal growth and sporulation were assessed and statistical analyses performed as described in 3.1.3.1.

3.1.3.4 Effect of inoculation technique and subculturing

Two inoculation procedures (*viz.* conidial and mycelial transfer), and five subculture treatments (*viz* 1st, 2nd, 3rd, 4th and 5th subcultures) were investigated. Subcultures were produced by transferring mycelial fragments to new media when the parent cultures were 21 days old. Conidial, and mycelial subcultures were produced on SSMA, and PDA, respectively.

Each subculture was inoculated onto SSMA plates using each of the two inoculation procedures (3 transfers per plate). Inoculated plates were then incubated as described in 3.1.3.1b.

Split plot experimental design was used with inoculation procedure, and subculturing as whole-plot, and sub-plot treatments respectively. Whole plots were arranged in a completely randomized design with 3 replications.

Each sub-plot comprised of 3-petri-plates.

Fungal growth and sporulation were assessed and statistical analyses performed as described in 3.1.3.1

3.2 Glasshouse Experiments

Studies in the glasshouse were conducted to test pathogenicity of *C. sesami* and *C. sesamicola* on *S. indicum* and related species of the crop, as well as to investigate histopathological relationships of the two fungi on susceptible sesame plants.

3.2.1 Tests for pathogenicity of *C. sesami* and *C. sesamicola* on *S. indicum* and on three wild species of *Sesamum*

3.2.1.1 Pathogenicity tests on *S. indicum*

This investigation was conducted to provide proof that isolates of the two fungi were pathogenic on *S. indicum.*

(a) Test plants

Pathogenicity tests on cultivated *S. indicum* were conducted using sesame plant accession SIK 134 (Greatest disease severity was observed on this accession during the preliminary survey exercise at Siaya FTC). Seeds of this accession were obtained from sesame improvement project of the University of Nairobi.

Seedborne inocula were eliminated prior to planting through treatment with 0.15% w/v Triadimefon (25% WP) to (Vyas, 1981). Treated seeds were sown in well drained eutric humic nitisol contained in 30-cm-diameter pots. Planting was done in time to provide 8-wk old plants at the time of inoculation.

(b) Inoculum preparation

Mycelial suspension was prepared from 21-day old monosporic cultures grown on PDA and the suspension spread on the surface of SSMA plates (1-ml per 9-cm diameter plate). The plates were incubated under normal room conditions for 14 days after which they were flooded with sterile distilled water (10-ml/plate). Conidia were dislodged by gently scrapping the surface using sterile glass rods and the suspension produced was strained through two layers of sterile cheese cloth. Conidial concentration in the suspension was determined using haemacytometer count (6 counts per suspension) and standardized at 2×10^4 conidia/ml.

(c) Inoculation of test plants

Eight-week old plants were inoculated using a modification of the technique described by Van der Vossen *et al.* (1976). Inocula was sprayed to run-off on both sides of all leaves present on plants using a 0.5-litre Baygon atomizer (Bayer East Africa Ltd.). A second inoculation was applied 48 hours later. Control plants were sprayed with sterile distilled water.
All inoculated plants were covered with moistened polythene bags and incubated in a shaded part of the greenhouse where temperatures ranged from 18 to 28^0 C. The polythene bags were removed after 6 days of incubation.

(d) Reisolation and culturing

Inoculated plants were examined daily for symptom development and colour, shape and size of lesions produced on the leaves recorded. Twenty eight days after inoculation, leaves showing characteristic disease symptoms were detached and reisolation of the causal fungus performed to fulfil Koch's postulates.

3.2.1.2 Pathogenicity tests on *S. angolense*, *S. calycinum*, and *S. latifolium*

This study was conducted to determine the host range of *C. sesami* and *C. sesamicola*.

(a) Test plants

Tests for pathogenicity of both fungi on wild species of *Sesamum* were conducted using *S. angolense* Welw., *S. calycinum* Welw. and *S. latifolium* Gillet. Seeds of these species were collected from Kibwezi in Machakos District and Marigat in Baringo District. Parts of the mature plants were also collected and taken to the Department of Botany, University of Nairobi, for species identification.

Dormancy in the collected seeds was broken using a modification of the technique described by Ashri and Palevitch (1976). The seeds were soaked in Gibberellic acid (300 ppm) for 24 hours and warmed in the same solution at 40^{0} C for 30 minutes. Seeds were then treated with 0.15% Triadimefon and sown in plastic pots as described in 3.2.1.1. Sowing was done in time to provide 10-wk old plants at the time of inoculation.

(b) Inoculation of test plants

Inoculum was prepared and 10-week old plants were inoculated and incubated as outlined in 3.2.1.1.

(c) Reisolation and culturing

Inoculated plants were observed daily for symptom appearance. Twenty eight days after inoculation, leaves showing infection symptoms were detached and reisolation of the two fungi carried out to establish pathogenicity.

3.2.2 Study of histopathological relationships of *C. sesami* and *C. sesamicola* on susceptible sesame

(a) Test plants

Histopathological studies were conducted using sesame plant accession SIK 134. Seeds of this accession were obtained from the sesame improvement project of the University of Nairobi.

Seeds were treated with 0.15% w/v Triadimefon (25% WP) to eliminate seedborne inocula prior to planting (Vyas, 1981). Treated seeds were sown as outlined in 3.2.1.1. Planting was done in time to provide 8-wk old plants at the time of inoculation.

(b) Inoculation of test plants

Inoculum was prepared and 8-wk old plants were inoculated and incubated as outlined in 3.2.1.1.

(c) Clearing and staining of leaf discs

The polythene bags were removed briefly after 1.5, 3, 6, 12, 24 and 48 hours and leaf tissue discs cut out using a 3-mm diameter cork borer. Leaf discs from each harvest were cleared by placing them in pyrex bottles containing Carnoy's solution (glacial acetic acid and absolute ethanol at a ratio of 1:2 v/v) for 24 hours. The cleared leaf discs were mounted in lactophenol-cotton blue on clean slides for microscopic observation.

(d) Determining mode of conidial germination

Stained leaf discs at each harvest were examined under the light microscope and the harvest time for leaf discs on which germination was first observed noted. Germinated conidia were examined to note the site of emergence of

the germ tubes as well as any fungal structure formed by the conidium prior to penetration of host leaf surface.

(e) Data collection

The time of harvest for leaf discs on which penetration of host tissue by fungal structures first occurred and the point at which the germ tube penetrated the leaf surface (*viz.* stomata or intact leaf surface) were noted.

Inoculated plants were examined daily for development of disease until 42 days after inoculation. The colour, shape, and size of lesions produced on all infected plant parts were noted.

3.3 Field experiments

Field studies were conducted to evaluate the relative susceptibility of 16 sesame accessions to white and angular leaf spots of sesame

(a) Inoculum preparation

Inoculum was prepared using a modification of the technique described by Robert and Findley (1952). Sesame plant materials showing severe symptoms attack by *C. sesami* and *C. sesamicola* were collected from the University of Nairobi's sesame seed multiplication nursery at Siava Farmers Training Centre (FTC) in June 1992. The materials were sorted out on the basis of the symptoms on them to separate the two fungi, and then dried at ambient temperature (20 to 26^0 C). The dry tissues were then chopped into small pieces and stored in sealed polythene bags placed in dark cupboards within the laboratory.

(b) Plant materials

Experimental materials were obtained from germplasm collection of the sesame improvement project of the University of Nairobi. These comprised of 16 sesame plant accessions *viz.* SIK 005, SIK 031(2), SIK 071, SIK 093, SIK 122, SIK 132, SIK 134, SPS 005, SPS 007, SPS 013, SPS 028, SPS 045, SPS

089, SPS 093 SPS 096 and SPS 113. Accessions used in the study were selected based on visual disease ratings made during a preliminary survey conducted at the University of Nairobi's seed multiplication nursery at Siaya FTC. Accessions were chosen to obtain an arbitrary range levels of resistance to *C. sesami* and *C. sesamicola*.

(c) Inoculation

Experimental materials were planted at Kibwezi Dryland Research Field Station (DRFS) in Machakos district, and Siaya Farmers Training Centre (FTC) in Siaya district on 2nd October, 1992, and 28th, October 1992, respectively. Ecological conditions at the two locations are presented in appendix 2.

Seeds of each accession were steeped in 0.15 % (w/v) Triadimefon (25% WP) before planting in the experimental fields. Plots consisted of two 6-m rows spaced 50-cm apart and were arranged along a strip of land perpendicular to the direction of the prevailing wind. Plots were separated from each other at the ends and sides by 3-m spreader strips planted with sesame accession SIK 134. Experimental fields for studying reaction of the accessions to *C. sesami* were enclosed by 2m strips of sorghum (tall landrace), to provide shade to the plants within the plot. (During the preliminary survey, greater severity to white leaf spot was observed on plants shaded by a hedge bordering one side of the field)

Inoculation was achieved through a modification of the technique described by Robert and Findley (1952). Preserved plant tissues harbouring inoculum were sprinkled along the planted rows (5g/row) 1-wk after sowing. Standard cultural practices for growing of sesame were followed at each experimental site, but no pesticides were applied.

(d) Experimental design

Randomized complete block design with three replications was used. Treatments included the 16 different accessions of sesame.

(e) **Determination of Area under disease progress curves (AUDPCs)**

These were computed from % infected leaves and % defoliation using the following formula (Shaner and Finney, 1977).

$$AUDPC = \sum [(Y_{i+1} + Y_i)/2] (X_{i+1} - X_i)$$

Where, Y_i = % disease severity (infected leaves or defoliation at the ith observation)

X_i = Date of ith observation in days after inoculation.

Percent infected leaves and percent defoliation were assessed as from November, 1991 to January, 1992 at Siaya FTC, and as from December, 1991 to February, 1992 at Kibwezi DRFS. The assessments were conducted using a modification of the technique described by Johnson and co-workers (1986).

To determine percent infected leaves, each row within the plot was divided into 60-cm sections prior to each assessment date. One of these 60-cm 'rating segments' per row was randomly selected every 10 to 14 days in each plot. The proportion of leaves containing at least one visible lesion within the selected rating segments was determined and used to compute % infected leaves on each observation date.

Percent defoliation was estimated by subdividing the selected rating segments into four 15-cm lengths. One of these 15-cm 'rating units' was randomly selected every 10 to 14 days and the number of nodes and missing leaves counted on each main stem within the unit. The proportion of leaves defoliated was calculated by dividing the number of missing leaves by twice the number of nodes.

(f) **Estimation of disease increase rates**

Gompertz and Logistic models were fitted to % infected leaves and % defoliation data. Apparent rates of disease increase were obtained by

regressing transformed disease data against time (expressed as days after planting).

(g) Statistical analysis

Averages for AUDPCs, infection rates and defoliation rates for each plot were examined using Analysis of Variance. Significant differences were identified using Duncan's multiple range test as applied by Luke and Berger (1982).

CHAPTER 4

RESULTS

4.1 Laboratory experiments: Colony features, morphological characteristics and effects of cultural conditions on *in vitro* growth and sporulation of *C. sesami* and *C. sesamicola*

4.1.1 Colony characteristics of *C. sesami* and *C. sesamicola*

C. sesami and *C. sesamicola* produced dark-grey mycelial colonies on PDA after 14 days of incubation (Plate 1). Colonies of *C. sesami* were large and spread laterally on culture media as opposed to those of *C. sesamicola* which were smaller and grew upwards. Cultures of both fungi that had been preserved as described in 1.1 could still be recovered after one year of storage. Colony morphology of the recovered isolates was similar to that of original isolates.

4.1.2 Morphological characteristics of *C. sesami* and *C. sesamicola*

Conidiophores of *C. sesami* olivaceous brown, unbranched, filiform, nodulose, 5 to 18 septate, and 180 to 370 by 5.5 to 10.0u (Plate 2). Each conidiophore carried 2 to 8 conidia. Conidia hyaline, subulate, basally truncate, apically obtuse, distinctly 6 to 22 septate and 120 to 280u by 3.5 to 6.0u. Each conidium had a distinct round, dark coloured ring on its basal end. Most conidia were slightly curved, although some straight forms were also observed. Some conidia had germinated by the 14th day of incubation to form germ tubes which fused by anastomosis (Plate 2).

Conidiophores of *C. sesamicola* hyaline to olivaceous brown, highly branched, irregularly shaped, nodulose, 3 to 9 septate, and measured 80 to

Plate 1. Cultures of *Cercospora sesami* (A) and *Cercospora sesamicola* (B) produced on potato dextrose agar 14 days after inoculation.

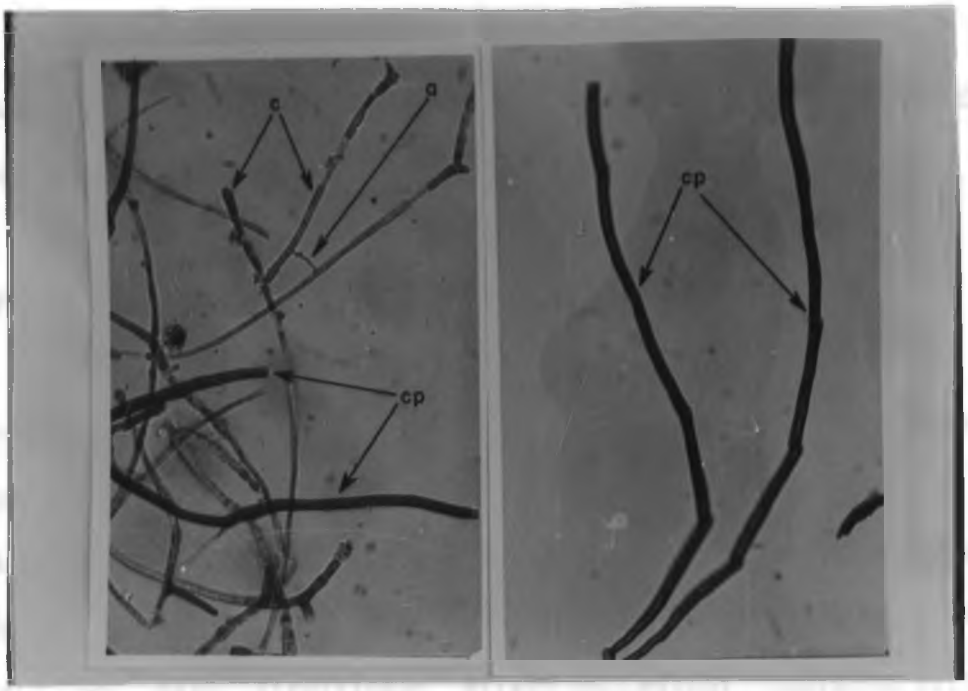

Plate 2. Conidia (c) and conidiophores (cp) of *Cercospora sesami* produced on sesame stem meal agar 14 days after inoculation. Note also the anastomosis (a) between germtubes of two adjacent conidia. Magnification x 620.

140u by 3 to 5u (Plate 3). Each conidiophore carried 1 to 7 conidia. Conidia hyaline, filiform, basally truncate, apically obtuse, indistinctly 3 to 8 septate, 95 to 150 by 3.0 to 4.5u and had a distinct round, dark coloured ring on their basal ends (Plate 3).

4.1.3 Factors influencing *in vitro* growth and sporulation of *C. sesami* and *C. sesamicola*

4.1.3.1 Effect of media composition and quantity

Growth and sporulation of both *C. sesami* and *C. sesamicola* occurred on all the thirteen media at each of the three media quantities tested (Tables 1 and 2). In both fungi, media composition (MC) and media quantity (MQ) had high significant effect on both fungi growth and sporulation, but the MCxMQ interaction did not have significant effect on either characteristic (Appendix 3).

Radial growth of *C. sesami* was greatest on potato dextrose agar (PDA) and carrot leaf decoction agar(CLDPDA), whereas that of *C. sesamicola* was optimum on CLDPDA. Least growth of both fungi occurred on corn meal agar (CMA). In both fungi, greatest sporulation was observed on sesame stem meal agar (SSMA), whereas smallest quantity of spores was produced on CMA. Colony diameters of the two fungi were significantly greater in plates containing 35-ml of media than in those carrying 15 or 25-ml. Sporulation of *C. sesami* was, however, significantly greater in plates bearing 15-ml than in those holding 25 or 35-ml of media. In contrast, *C. sesamicola* produced the most abundant quantity of conidia in plates containing 35-ml of media.

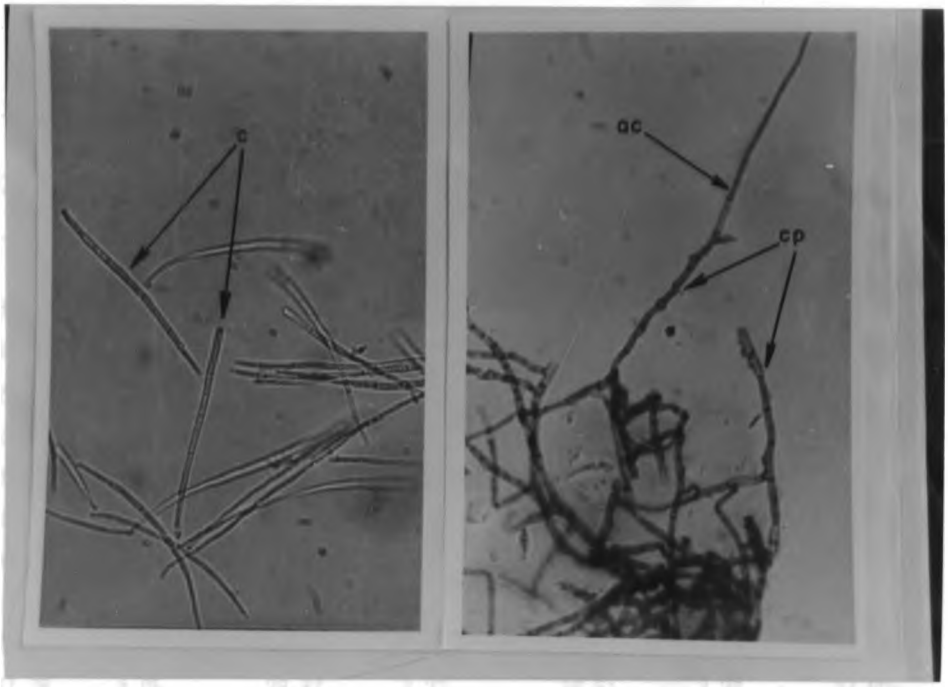

Plate 3. Conidia (c) and conidiophores (cp) of *Cercospora sesamicola* produced on sesame stem meal agar 14 days after inoculation. Note also the attachment of conidium to conidiophore (ac). Magnification x 840.

Table 1. Growth and sporulation of *Cercospora sesami* on thirteen media as influenced by media quantity 21 days after inoculation

	Media quantity							
	15-ml		25-ml		35-ml		Mean[a]	
Medium	CD[b]	CC[b]	CD	CC	CD	CC	CD	CC
CLDA	37.41	7.64	38.02	6.09	39.66	6.19	38.36b	6.64bc
CLDPDA	41.07	3.64	41.48	2.90	43.65	2.95	42.07a	3.16de
CMA	21.79	1.15	21.85	1.14	22.84	0.99	22.16f	1.09f
MEA	23.49	4.53	23.44	4.19	25.53	3.88	24.15f	4.20d
OA	30.10	4.57	30.56	3.63	31.36	3.70	30.67d	3.97de
PCA	35.04	6.95	36.09	5.53	37.79	5.64	36.31bc	6.04c
PDA	41.05	2.78	41.56	2.21	43.47	2.25	42.03a	2.42def
SLDA	15.48	8.48	15.62	6.75	17.28	4.53	16.13g	6.59bc
SLDOA	15.31	2.58	16.00	2.05	17.15	2.09	16.15g	2.24ef
SLDPDA	27.78	4.05	26.77	3.22	28.51	3.28	27.69e	3.52b
SSMA	33.20	13.32	36.17	12.00	36.22	11.24	35.20c	12.19a
SSMOA	27.10	8.58	28.78	8.03	32.20	7.37	29.36de	7.99b
SSMPDA	34.60	4.25	32.27	4.18	36.57	3.71	34.48c	4.05de
Mean[c]	29.49	5.58	29.89	4.76	31.71	4.45		

[a] Average of means over the 3 media quantities; values followed by the same letter do not differ significantly at p=0.01 (Duncan's multiple range test).

[b] CD= Colony diameter (mm), CC= Conidial concentration (x1000 conidia/ml). Each value is the average of three replications.

[c] Average of means over the 13 types of media; LSD at p= 0.01 for comparing colony diameter, and conidial concentration values are 0.85-mm, and 0.45 x 10^3 conidia/ml, respectively.

Table 2. Growth and sporulation of *Cercospora sesamicola* on thirteen media as influenced by media quantity 21 days after inoculation

	Media quantity							
	15-ml		25-ml		35-ml		Mean[a]	
Medium	CD [b]	CC [b]	CD	CC	CD	CC	CD	CC
CLDA	3.65	4.69	3.74	4.86	3.96	5.29	3.78bcd	4.95cd
CLDPDA	4.97	5.15	5.07	5.35	5.39	5.82	5.14a	5.44cd
CMA	1.34	1.39	1.37	0.38	1.45	7.09	1.45g	0.40e
OA	3.70	2.39	3.80	2.47	4.02	2.70	3.84bcd	2.52de
MEA	3.54	6.30	3.63	6.49	3.83	7.09	3.67bcde	6.62c
PCA	3.16	4.30	3.25	4.56	3.44	4.89	3.28def	4.59cd
PDA	3.76	4.29	3.85	4.46	4.07	4.85	3.89bc	4.53cd
SLDA	2.89	14.30	2.96	14.64	3.13	16.15	2.99f	15.03b
SLDOA	3.04	5.59	3.11	8.96	3.30	9.76	3.15ef	8.10c
SLDPDA	3.39	7.12	3.47	7.45	3.68	8.05	3.51cdef	7.54c
SSMA	3.71	25.03	3.79	25.98	4.01	28.27	3.84bcd	26.43a
SSMOA	3.88	11.72	3.97	11.96	4.21	13.10	4.02bc	12.26b
SSMPDA	4.00	11.20	4.10	11.59	4.34	12.68	4.15b	11.82b
Mean [c]	3.46	7.88	3.55	8.40	3.76	9.16		

[a] Average of means over the 3 media quantities; values followed by the same letter do not differ significantly at p=0.01 (Duncan's multiple range test).

[b] CD= Colony diameter (mm), CC= Conidial concentration (x1000 conidia/ml). Each value is the average of three replications.

[c] Average of means over the 13 types of media; LSD at p= 0.01 for comparison of colony diameter, and conidial concentration values are 0.11-mm, and 0.71 x 10^3 conidia/ml, respectively.

4.1.3.2 Effect of incubation period and media pH

Results showing the effects of incubation period on mycelial growth and sporulation of *C. sesami* and *C. sesamicola* at each of the six pH levels tested are presented in tables 3 and 4. Incubation period (IP), media pH (MP) and the IPxMP interaction had highly significant effects on growth and sporulation of both fungi (Appendix 4)

Mycelial growth of the two fungi increased with prolonged incubation through the 28th day at all the pH levels (Figs. 1 and 2). The largest amount of mycelium of *C. sesami* was produced at pH 6.48 for all the incubation periods investigated. Growth of *C. sesami* was significantly greater at the optimum pH than at the other tested pH levels for all durations of incubation except 28 days. In *C. sesamicola*, the optimum pH for growth varied with different durations of incubation. On incubating cultures of this fungus for 8 days, maximum growth occurred at pH 6.48. Incubation of the fungus for longer durations gave optimum growth at pH 7.00. Growth of *C. sesamicola* at the optimum pH levels was significantly greater than that at the other pH levels except pH 7.00, pH 6.48, pH 6.00 and 6.48, and pH 6.00, 6.48 and 8.00 in cultures incubated for 7, 14, 21, and 28 days, respectively..

Prolonged incubation also increased sporulation through the 28th day at pH 9 in *C. sesami*, and at all the six pH levels in *C. sesamicola*. At pH levels below 9.00, sporulation of *C. sesami* increased with prolonged incubation until the 21st day, but declined thereafter (Figs. 3 and 4). Most spores of *C. sesami*, and *C. sesamicola* had germinated by 21st day of incubation to give vegetative hyphae, and secondary conidia, respectively. The optimum pH for sporulation of *C. sesami* was dependent on the duration of incubation. On incubating cultures of this fungus for 7 days, greatest quantity of conidia were obtained at pH 6.00. Upon incubating the fungus for longer durations, the most abundant quantity of conidia were produced at pH 6.00. The amount of conidia produced by *C. sesami* was significantly

Table 3. Growth and sporulation of *Cercospora sesami* on sesame stem meal suspension as affected by interaction of pH and incubation period

Media pH	Incubation period									
	MDW [b]	CC [b]	MDW	CC	MDW	CC	MDW	CC	MDW	CC
5.00	18.43cd	8.09abc	38.10cd	8.94bc	72.00bc	12.69b	87.80ab	10.64bc	54.08	10.09
6.00	26.97b	9.18a	43.70b	11.32a	74.00b	17.53a	89.70ab	12.39a	58.59	12.61
6.48	36.40a	8.71ab	51.47a	12.39a	80.33a	18.10a	91.57a	12.44a	64.94	12.91
7.00	22.87bc	7.74abc	42.00bc	9.50b	71.60bc	17.54a	88.63a	11.13ab	56.28	11.48
8.00	17.23de	7.25bc	35.77d	8.11bc	69.60bc	10.25c	87.30a	9.40c	52.48	8.75
9.00	13.07e	6.45c	33.17d	7.79c	66.93c	9.09c	85.67ab	9.13c	49.71	8.11
Mean	22.49	7.90	40.70	9.68	72.41	14.20	88.44			

[a] Mean mycelial growth (or conidial production) per day

[b] MDW= mycelial dry weight (mg) and CC= Conidial concentration (x1000 conidia/ml). Each value is the average of three replications. Within each incubation period, means followed by the same letter do not differ significantly at p= 0.01 (Duncan's multiple range test). At each pH, LSD at p= 0.01 for comparison of mycelial dry weight, and conidial production means are 5.55-mm, and 0.59×10^3 conidia/ml, respectively.

Table 4. Growth and sporulation of *Cercospora sesamicola* on sesame stem meal suspension as affected by interaction of pH and incubation period.

	Incubation period									
	7 days		14 days		21 days		28 days		Mean [a]	
Media pH	MDW [b]	CC [b]	MDW	CC	MDW	CC	MDW	CC	MDW	CC
5.00	26.70d	7.41a	37.53c	13.02ab	71.93c	22.56b	86.90b	30.30a	55.77	18.32
6.00	31.53bc	9.20a	49.70b	14.11a	83.13ab	25.30ab	91.60a	32.64a	63.99	20.31
6.48	39.63a	10.19a	53.70ab	15.37ab	86.53a	26.80a	92.80a	26.80a	68.17	21.15
7.00	39.40a	8.06a	56.40a	12.29bc	87.00a	21.96b	93.83a	30.95a	67.99	18.32
8.00	27.73cd	7.74a	50.30b	10.34c	81.73b	17.90c	89.17ab	28.75a	62.23	16.18
9.00	23.90d	7.26a	31.50d	7.26d	67.00d	10.27d	84.60b	28.75a	51.75	13.38
Mean	30.73	8.31	46.52	12.07	79.56	20.80	89.82	30.93	61.66	18.03

[a] Mean mycelial growth (or conidial production) per day

[b] MDW= mycelial dry weight (mg) and CC= Conidial concentration (x1000 conidia/ml). Each value is the average of three replications. Within each incubation period, means followed by the same letter do not differ significantly at p=0.01 (Duncan's multiple range test). At each pH, LSD at p= 0.01 for comparison of mycelial dry weight, and conidial concentration means are 4.69-mg, and $1.68x10^3$ conidia/ml, respectively.

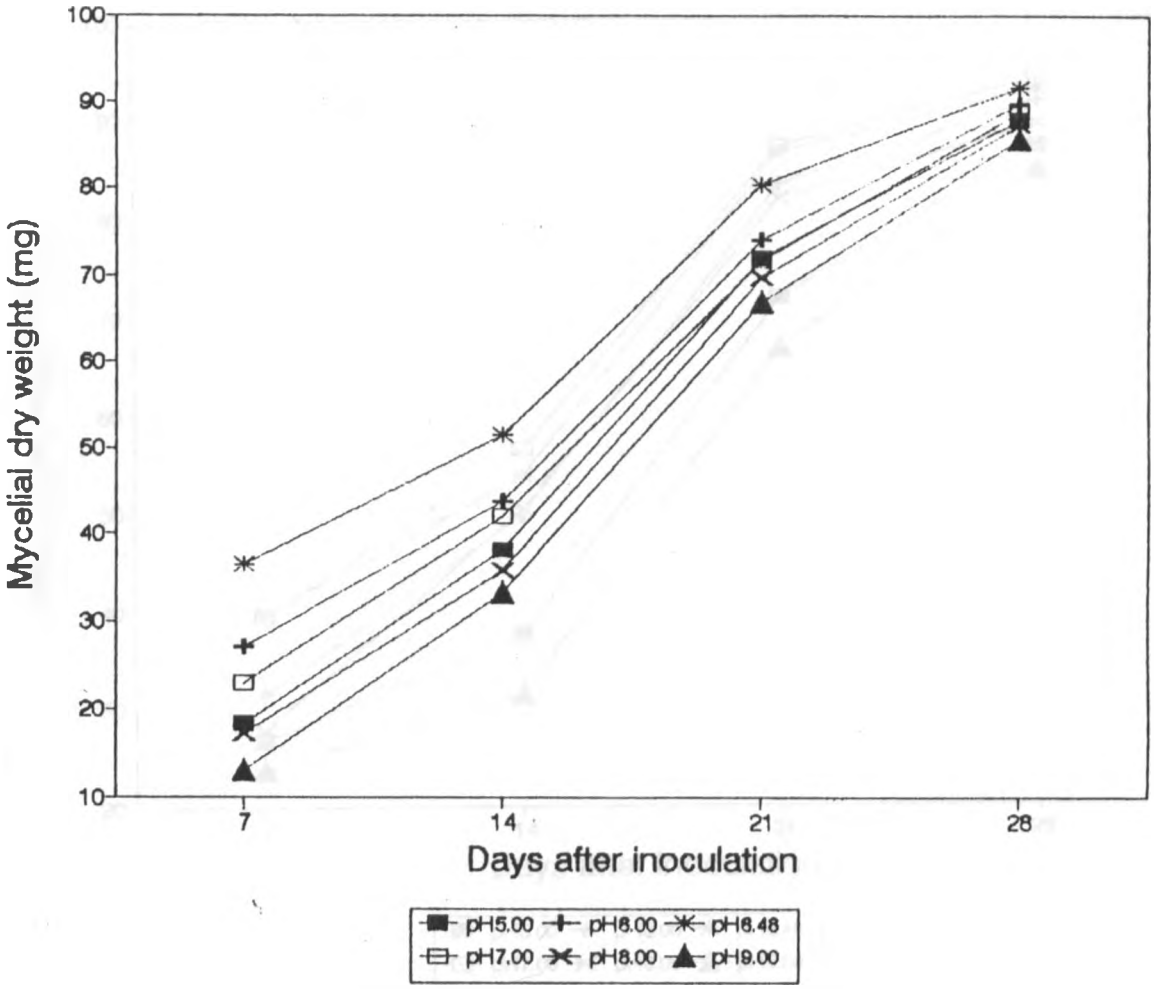

Fig. 1. Effect of incubation period on growth of *Cercospora sesami* at 6 pH levels of sesame stem meal suspension. (Mean separation in table 3).

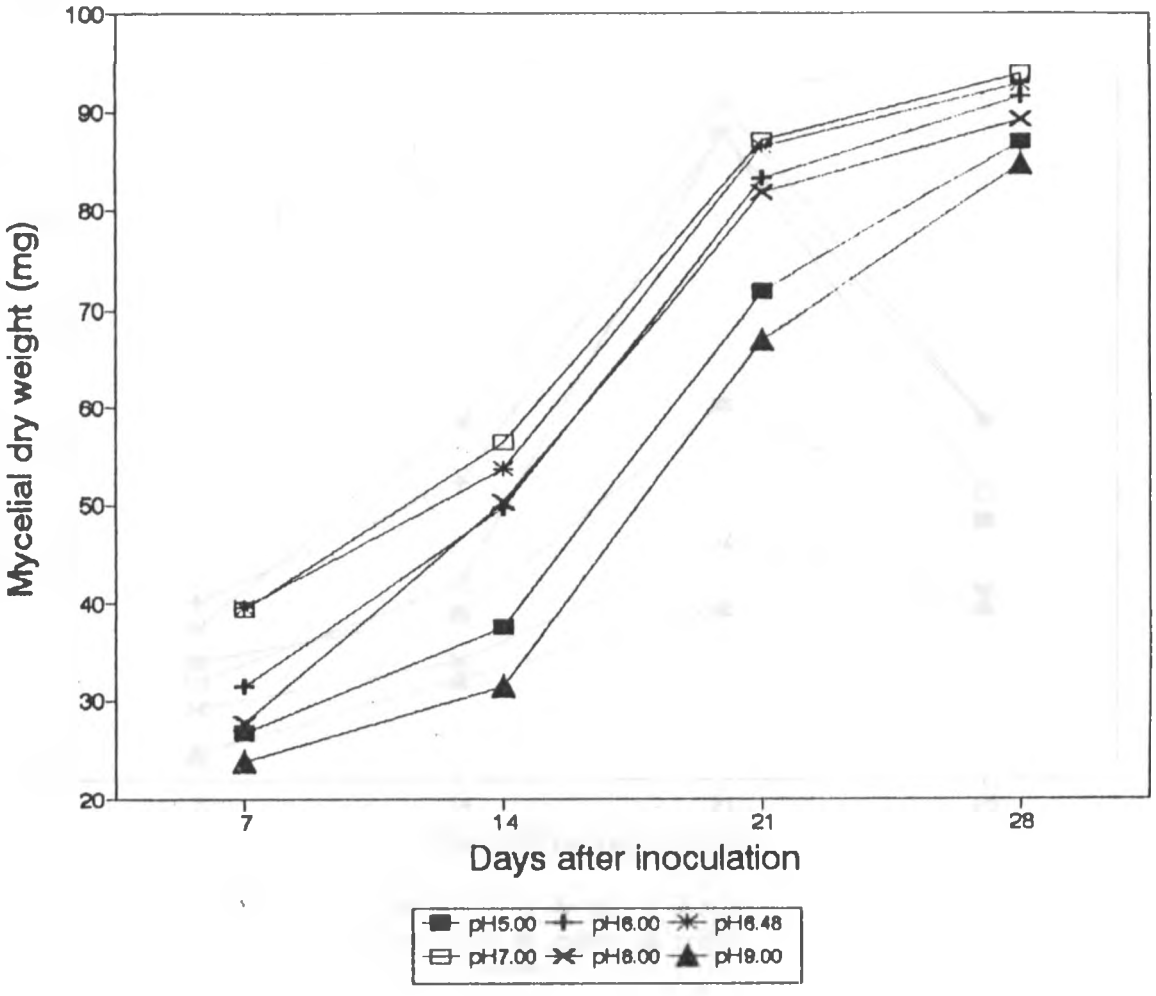

Fig. 2. Effect of incubation period on growth of *Cercospora sesamicola* at 6 pH levels of sesame stem meal suspension. (Mean separation in table 4).

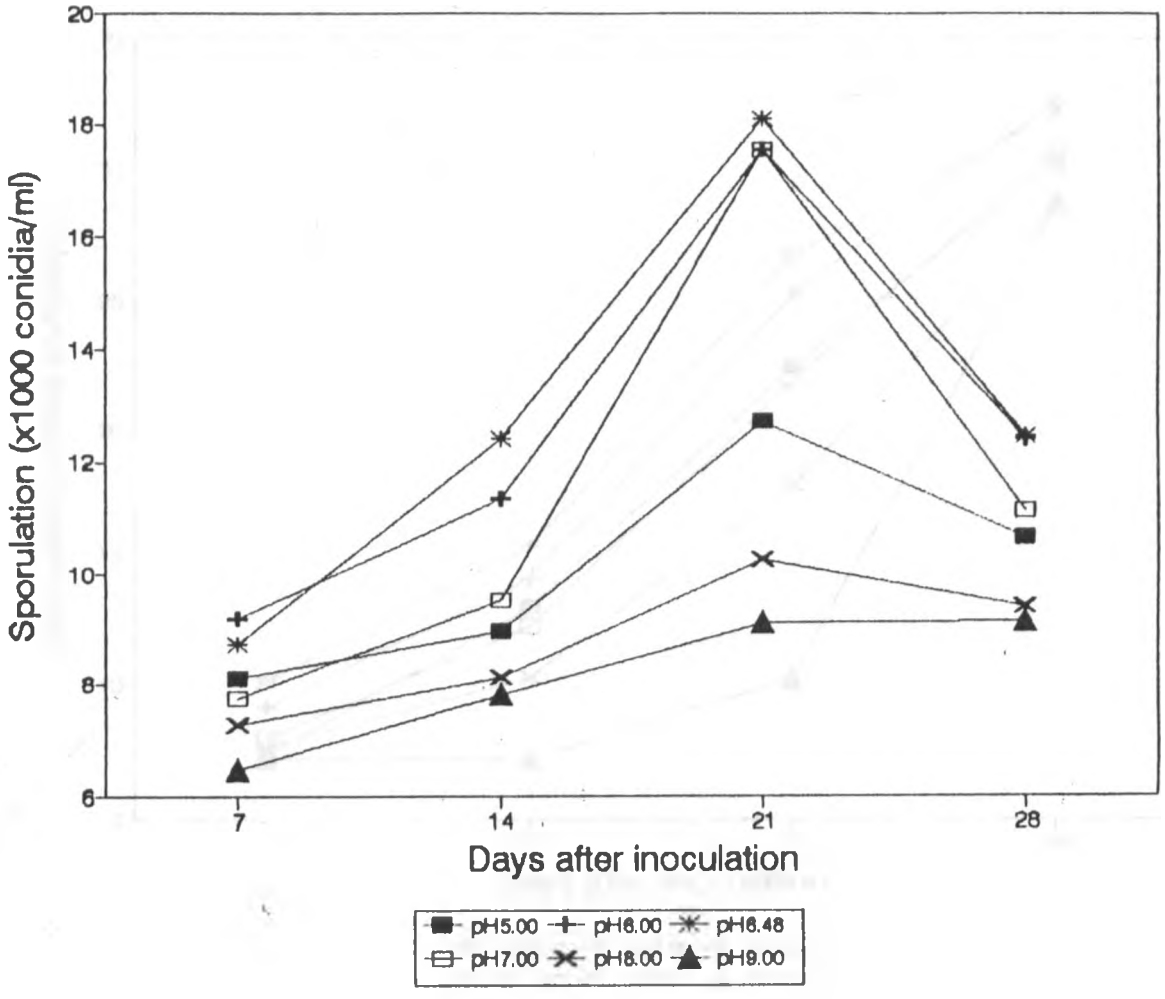

Fig. 3. Effect of incubation period on sporulation of *Cercospora sesami* at 6 pH levels of sesame stem meal suspension. (Mean separation in table 3).

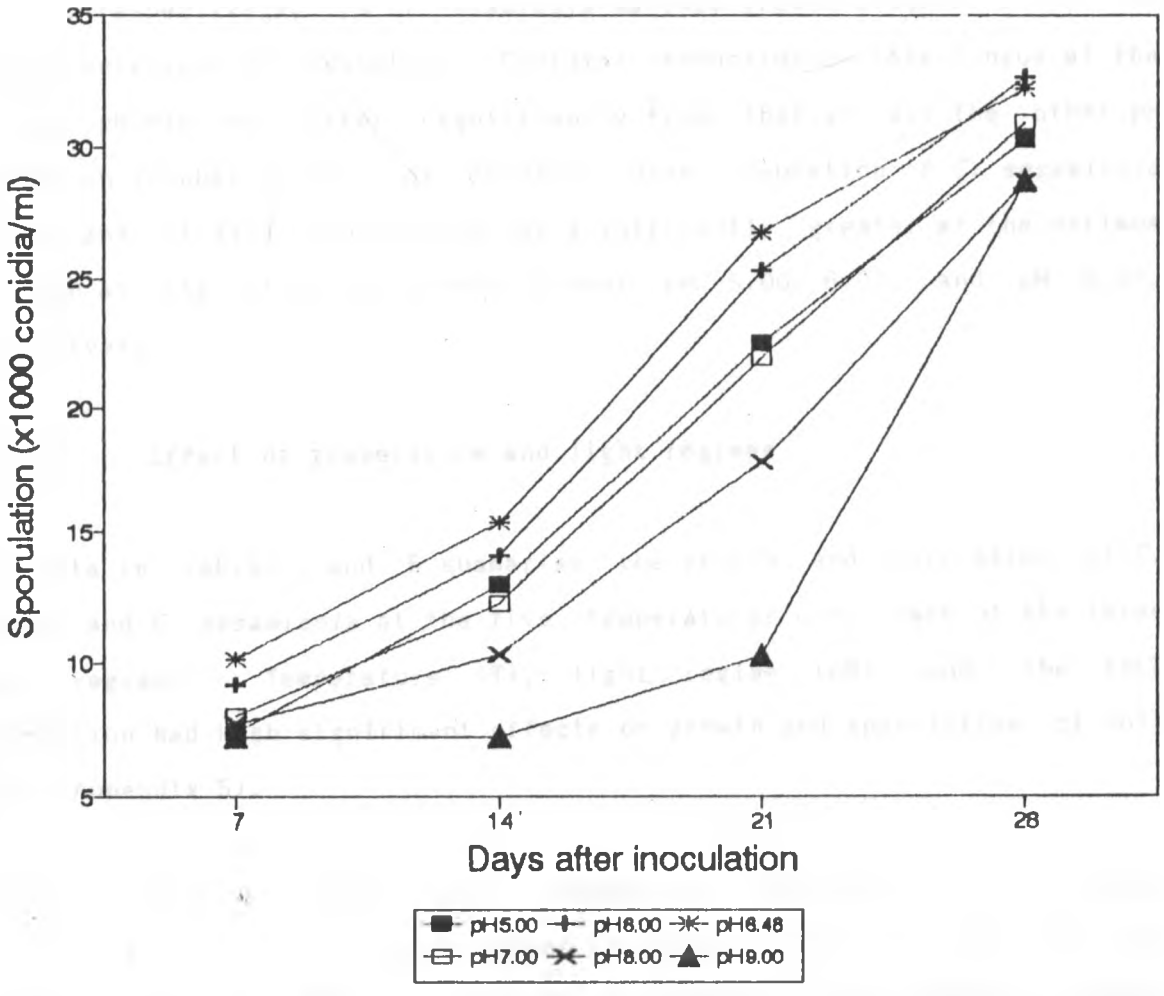

Fig. 4. Effect of incubation period on sporulation of *Cercospora sesamicola* at 6 pH levels of sesame stem meal suspension. (Mean separation in table 4).

the optimum pH levels than at the other pH levels except pH 5.00, 6.48, and 7.00, pH 6.00, and pH 6.00 and 7.00 on incubation for 7, 14 and more than 14 days, respectively. In *C. sesamicola* maximum growth occurred at pH 6.48 for all durations of incubation. Conidial production by this fungus at the optimum pH did not differ significantly from that at all the other pH levels on incubation for 7 or 28 days. Upon incubation of *C. sesamicola* for 14, and 21 days, sporulation was significantly greater at the optimum pH than at the other pH levels except pH 5.00, 6.00, and pH 6.00, respectively.

4.1.3.3 Effect of temperature and light regimes

The data in tables 5 and 6 summarise the growth and sporulation of *C. sesami* and *C. sesamicola* at the five temperatures under each of the three light regimes. Temperature (T), light regime (LR), and the TxLR interaction had high significant effects on growth and sporulation of both fungi (Appendix 5).

Growth of both *C. sesami* and *C. sesamicola* increased with increasing temperature until 25 ^{0} C, and declined thereafter under all the three light regimes (Figs. 5 and 6). In both fungi, optimum light regime for radial growth was dependent on the temperature. At temperatures below 35^{0} C (*viz* 15, 20, 25 and 30 ^{0} C), radial growth of the two fungi was greatest under continuous light. Colonies were significantly larger under this light regime than under continuous dark except at 20^{0} C, and at 15^{0} C, respectively. However the amount of growth of both fungi under continuous light did not differ significantly from that under alternating light/dark cycle except at 15 and 25 ^{0} C in cultures of *C. sesami*. At 35^{0} C, maximum radial growth of the two fungi was obtained under continuous dark. Although growth of both fungi under this light regime was significantly greater than that under continuous light, it did not differ significantly from that under alternating light/dark cycle.

Table 5. Growth and sporulation of *Cercospora sesami* on sesame stem meal agar as affected by interaction between light and temperature 21 days after inoculation

	Light regime							
	Cont. light[a]		Cont. dark[a]		Alt. light/dark[a]		Mean	
Temp. (° C)	CD[b]	CC[b]	CD	CC	CD	CC	CD	CC
15	12.30bc	5.37a	5.12c	6.00bc	8.67c	7.87b	8.69	6.41
20	15.41b	8.22a	14.15b	9.47ab	15.39b	10.20ab	14.98	9.30
25	35.11a	7.87a	22.13a	11.44a	26.81a	13.14a	28.02	10.82
30	10.02c	1.05b	7.95c	2.73cd	9.19c	1.52c	9.05	1.77
35	6.09c	0.73b	7.75c	1.61d	7.57c	1.54c	7.14	1.29
Mean	15.79	4.65	11.42	6.25	13.52	6.85		

[a] Cont. light= continuous light; cont. dark= continuous dark; and alt. light/ dark= 12-hr alternating light/dark cycles.

[b] CD= colony diameter (mm); CC= conidial concentration (x1000 conidia/ml). Each value is the average of three replications. Within each light regime, means followed by the same letter do not differ significantly at p=0.01 (LSD-test). At each temperature, LSD at p= 0.01 for comparison of colony diameter, and conidial concentration means are 1.35-mm, and 1.91×10^{3} conidia/ml, respectively.

Table 6. Growth and sporulation of *Cercospora sesamicola* on sesame sten meal agar as affected by interaction between light and temperature 21 days after inoculation

	Light regime							
	Cont. light[a]		Cont. dark[a]		Alt. light/dark[a]		Mean	
Temp. (^{o} C)	CD[b]	CC[b]	CD	CC	CD	CC	CD	CC
15	1.72d	10.15d	1.44c	9.36c	1.46d	9.69c	1.54	9.73
20	4.64b	14.25bc	3.33b	12.24	4.13b	12.47b	4.03	12.99
25	6.51a	25.09a	5.57a	18.18a	5.96a	17.30a	6.01	20.19
30	2.94c	15.12b	1.83c	11.89b	2.75c	12.33b	2.51	13.11
35	0.96d	12.77c	1.27c	11.25bc	1.14d	12.00b	1.12	12.01
Mean	3.35	15.48	2.69	12.41	3.09	12.93		

[a] Cont. light= continuous light; cont. dark= continuous dark; and alt. light/ dark= 12-hr alternating light/dark cycles.

[b] CD= colony diameter (mm); CC= conidial concentration (x1000 conidia/ml). Each value is the average of three replications. Within each light regime, means followed by the same letter do not differ significantly at p=0.01 (LSD-test). At each temperature, LSD at p= 0.01 for comparison of colony diameter, and conidial concentration means are 0.58-mm, and 1.47x10 ^{3} conidia/ml, respectively.

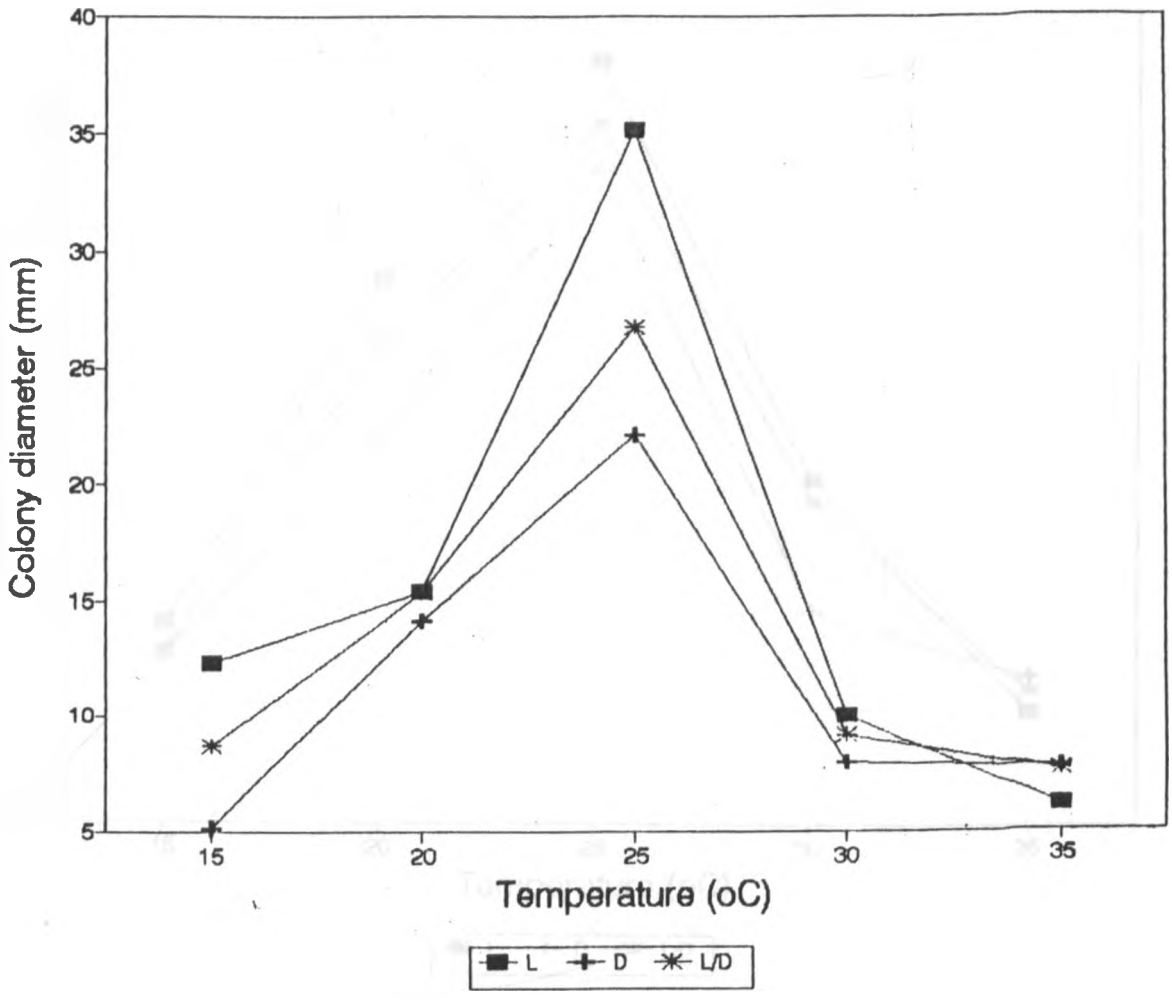

Fig. 5. Growth of *Cercospora sesami* on sesame stem meal agar as influenced by interaction of temperature and light regime 21 days after inoculation. L= continuous light, D= continuous dark, and L/D= 12-hr alternating light/dark cycles. (Mean separation in table 5).

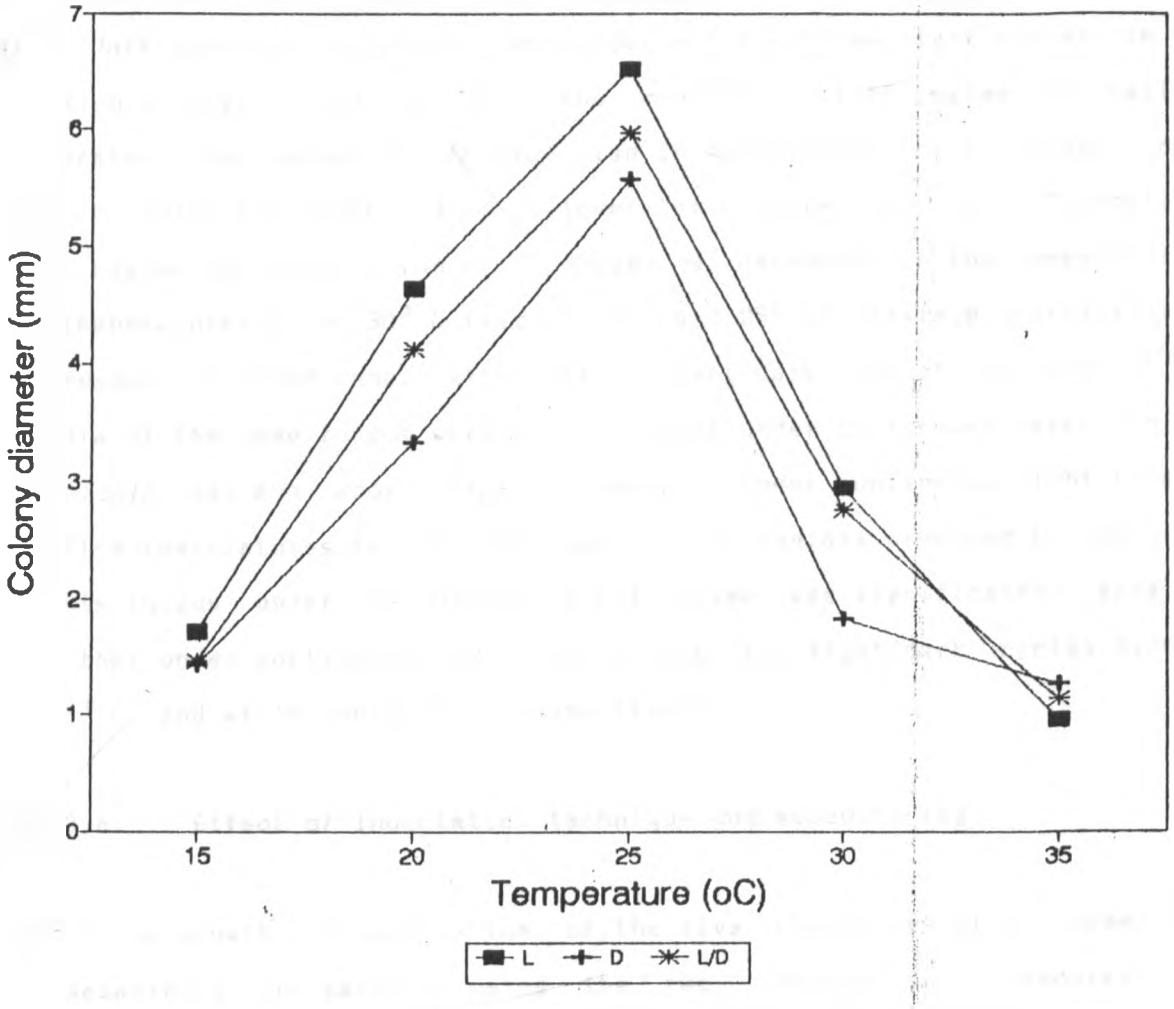

Fig. 6. Growth of *Cercospora sesamicola* on sesame stem meal agar as influenced by interaction of temperature and light regime 21 days after inoculation. L= continuous light, D= continuous dark, and L/D= 12-hr alternating light/dark cycles. (Mean separation in table 6).

Conidial production increased with increasing temperature until 25 ^{0} C but declined thereafter under only continuous darkness and alternating light/dark cycle in *C. sesami*, and under all the three light regimes in *C. sesamicola* (Figs. 7 and 8). Under the continuous light regime, increasing temperature also caused an initial rise in sporulation of *C. sesami*, but the declining trend started at a lower temperature (20 ^{0} C). The optimum light regime for sporulation of *C. sesami* was dependent on the temperature. At temperatures below 30^{0} C (*viz* 15, 20, and 25^{0} C), maximum sporulation of *C. sesami* occurred under alternating light/dark but at 30 and 35 ^{0} C, conidia of the same fungus were most abundant under continuous dark. In *C. sesamicola*, maximum sporulation was observed under continuous light at all the five temperatures tested. The quantity of conidia produced by cultures of this fungus under the optimum light regime was significantly greater than that under continuous dark, and alternating light/dark cycles except at 15^{0} C, and at 15 and 35 ^{0} C, respectively.

4.1.3.4 Effect of inoculation technique and subculturing

Results on Growth and sporulation of the five subcultures of *C. sesami* and *C. sesamicola* propagated using the two inoculation procedures are summarised in tables 7 and 8. Inoculation technique (IT) and subculturing (S) had highly significant effect on growth of both *C. sesami* and *C. sesamicola*, but the ITxS interaction produced significant effect on growth of only *C. sesamicola* (Appendix 6). Only inoculation technique and the ITxS interaction, and inoculation technique and subculturing had significant effects on sporulation of *C. sesami*, and *C. sesamicola*, respectively (Appendix 6).

Radial growth of both *C. sesami* and *C. sesamicola* changed with successive subculturing through the 5th subculture with both inoculation procedures (Figs. 9 and 10). Among all the five subcultures of *C. sesami* and

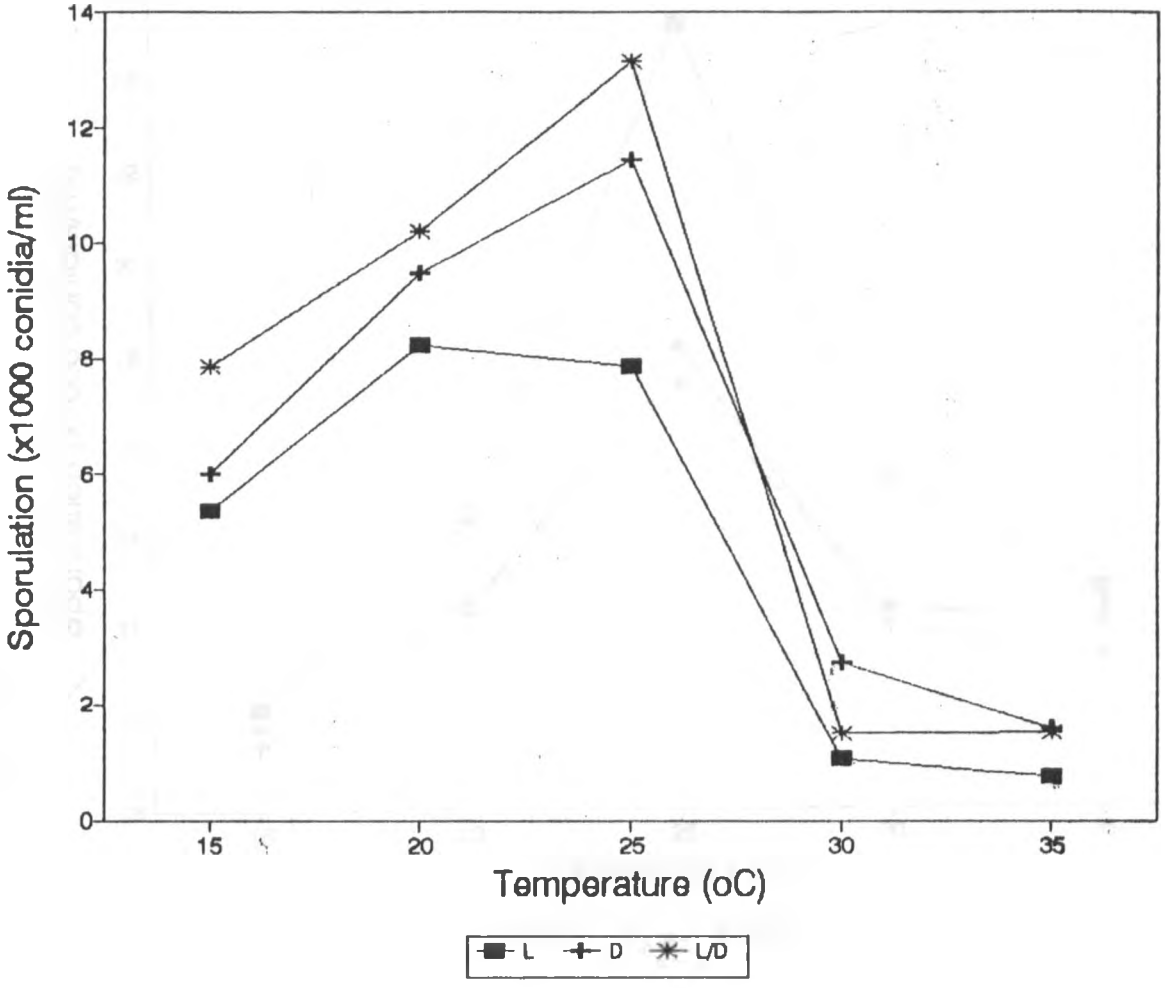

Fig. 7. Effect of interaction of temperature and light regime on sporulation of *Cercospora sesami* on sesame stem meal agar 21 days after inoculation. L= continuous light, D= continuous dark, and L/D= 12-hr alternating light/dark cycles. (Mean separation in table 5).

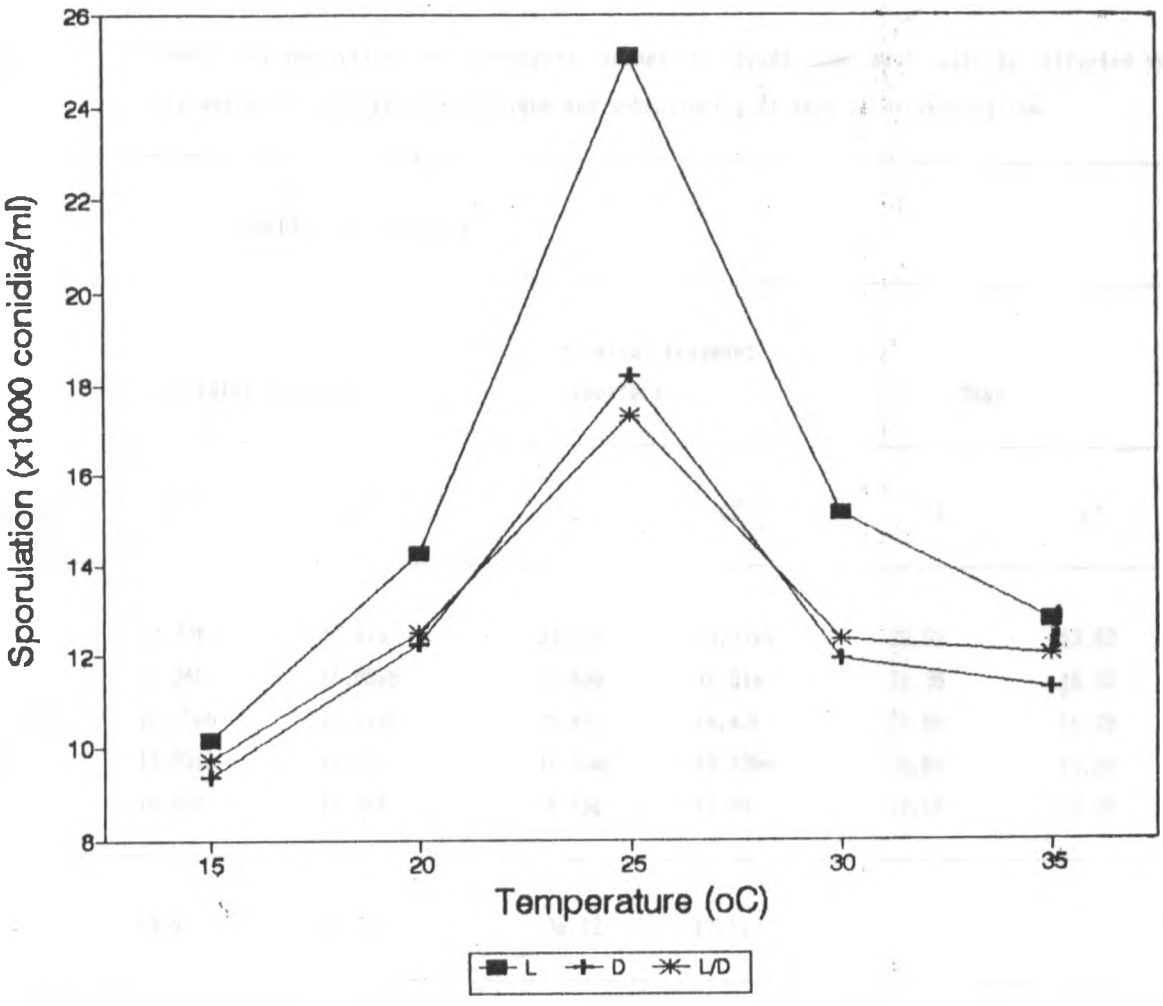

Fig. 8. Effect of interaction of temperature and light regime on sporulation of *Cercospora sesamicola* on sesame stem meal agar 21 days after inoculation. L= continuous light, D= continuous dark, and L/D= 12-hr alternating light/dark cycles. (Mean separation in table 6).

Table 7. Growth and sporulation of *Cercospora sesami* on sesame stem meal agar as affected by interaction of inoculation technique and subculturing 21 days after inoculation

	Inoculation procedure					
	Conidial transfers		Mycelial fragment transfers		Mean	
Subculture	CD[a]	CC[a]	CD	CC	CD	CC
1	12.39b	13.87b	33.24c	13.37ab	22.82	13.62
2	13.24b	15.89ab	35.63b	14.81a	24.36	15.35
3	13.75ab	17.15ab	35.49b	14.43a	24.69	15.79
4	13.83ab	17.92a	37.53ab	10.19bc	25.68	14.06
5	16.43a	17.95a	38.73a	12.09	27.58	13.59
Mean	13.93	16.56	36.12	12.41		

[a] CD= colony diameter (mm), and CC= conidial concentration (x1000 conidia/ml). Each value is the average of five replications. Within inoculation technique, means followed by the same letter do not differ significantly at p=0.01 (LSD). In each subculture, LSD at p= 0.01 for comparison of colony diameter, conidial concentration means are 3.67-mm, and 5.03×10^3 conidia/ml, respectively.

Table 8. Growth and sporulation of *Cercospora sesamicola* on sesame stem meal agar as influenced by inoculation technique and subculturing 21 days after inoculation

	Inoculation procedure					
	Conidial transfers		Mycelial fragment transfers		Mean	
Subculture	CD[a]	CC[a]	CD	CC	CD	CC
1	2.71b	27.19c	3.33c	25.12c	3.28c	26.16c
2	2.92a	29.18bc	3.85c	25.33bc	3.13c	26.76c
3	3.65a	29.24abc	5.44b	26.46abc	4.61	27.85bc
4	3.77a	30.23ab	5.86b	27.72ab	4.76b	28.98ab
5	3.93a	31.60a	7.46a	28.50a	5.69a	30.05a
Mean[c]	3.40	29.29	5.19	26.63		

[a] CD= colony diameter (mm), and CC= conidial concentration (x1000 conidia/ml). Each value is the average of five replications. Within each inoculation procedure, means followed by the same letter do not differ significantly at p= 0.01 (Duncan's multiple range test). In each subculture, LSD at p= 0.01 for comparing colony diameter, and sporulation means are 0.713-mm, and 3.51×10^3 conidia/ml, respectively.

[b] Average of colony diameter and conidial concentration means between the two inoculation procedures; values followed by the same letter do not differ significantly at p= 0.01 Duncan's multiple range test (DMRT).

[c] Average of colony diameter and conidial concentration means over the 5 subcultures. LSD at p= 0.01 for comparing colony diameter, and sporulation values are 0.71-mm, and 3.01×10^3 conidia/ml, respectively.

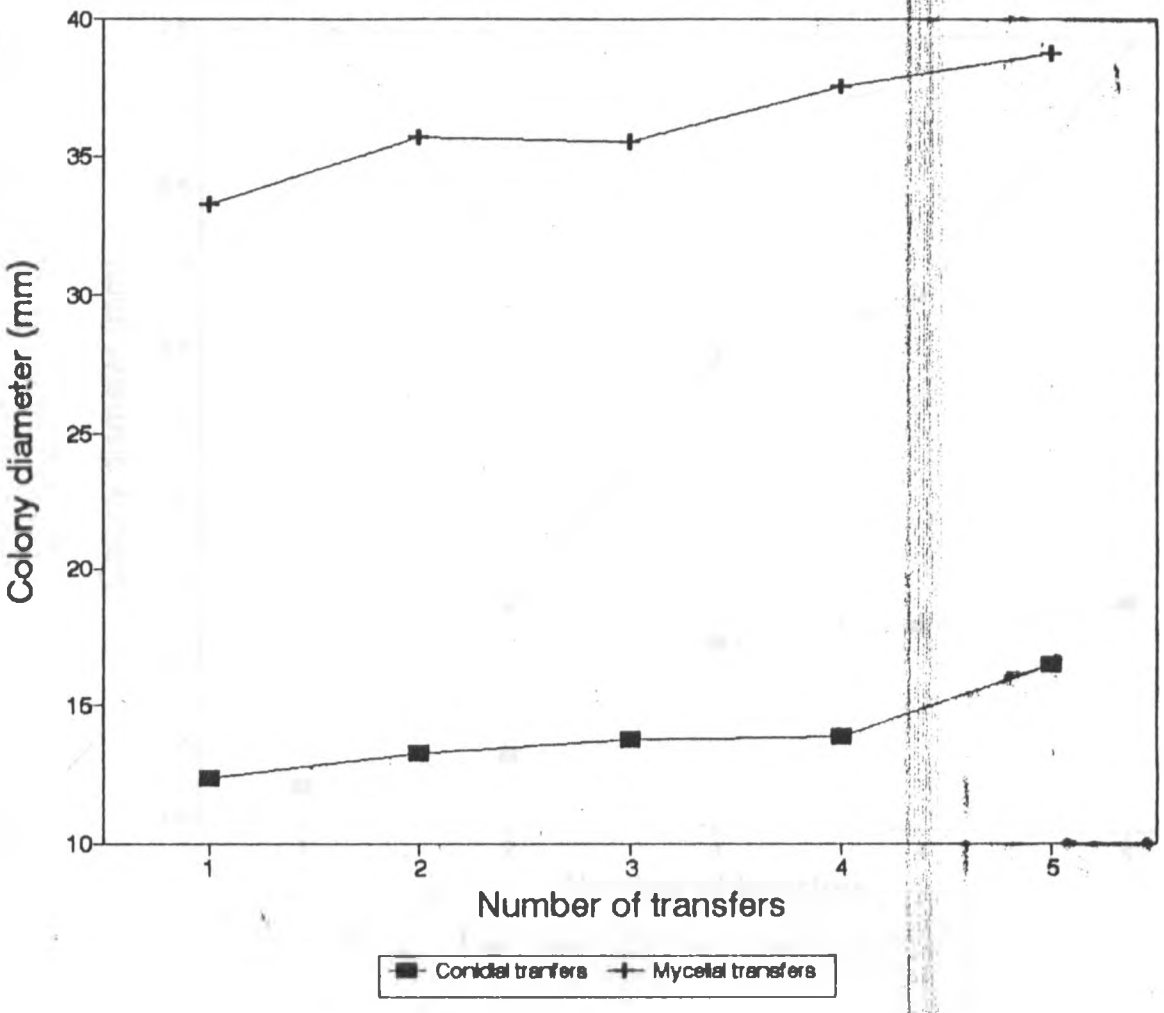

Fig. 9. Effect of inoculation technique and subculturing on growth of *Cercospora sesami* on sesame stem meal agar 21 days after inoculation. (mean separation in table 7).

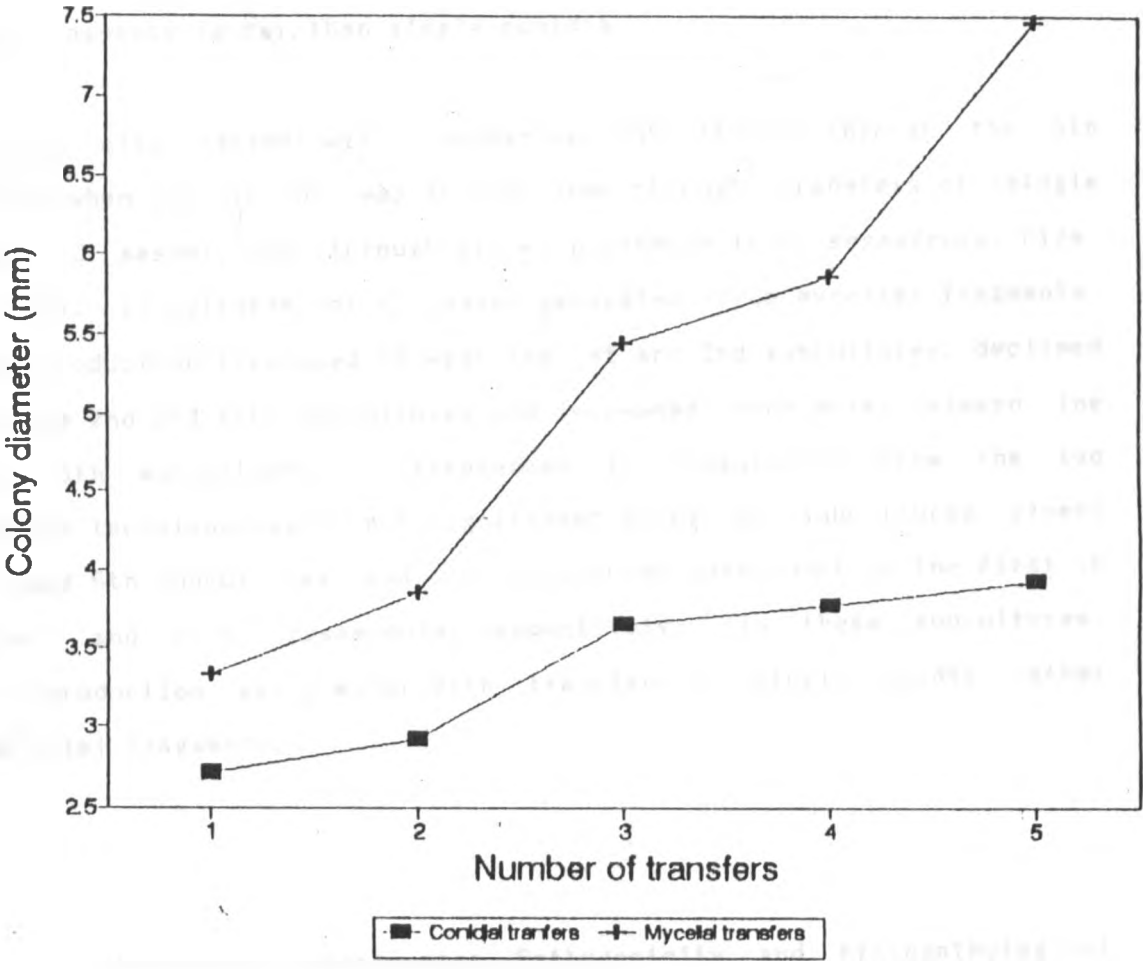

Fig. 10. Growth of *Cercospora sesamicola* on sesame stem meal agar 21 days after inoculation as influenced by interaction of inoculation technique and subculturing. (mean separation in table 8).

in subcultures of *C. sesamicola* subsequent to the first, colonies were significantly larger when inoculation was achieved through transfer of mycelial fragments rather than single-conidia.

Sporulation also varied with successive subculturing through the 5th subculture when inoculation was accomplished through transfers of single conidia in *C. sesami*, and through either procedure in *C. sesamicola* (Figs. 11 and 12). In cultures of *C. sesami* generated from mycelial fragments, conidial production increased between the 1st and 2nd subcultures, declined between the 2nd and 4th subcultures and increased, once more, between the 4th and 5th subcultures. Differences in sporulation from the two inoculation techniques were not significant among all subcultures except the 4th and 5th subcultures, and the subcultures subsequent to the first in *C. sesami*, and in *C. sesamicola*, respectively. In these subcultures, conidial production was greater with transfers of single conidia, rather than mycelial fragments.

4.2 Glasshouse experiments: Pathogenicity and histopathological relationships of *C. sesami* and *C. sesamicola*

4.2.1 Pathogenicity of *C. sesami* and *C. sesamicola* on *S. indicum*, and on three wild species of *Sesamum*

4.2.1.1 Pathogenicity of *C. sesami* and *C. sesamicola* on *S. indicum*

Within 12 to 28 days after inoculation, leaves of all plants inoculated with *C. sesami*, and *C. sesamicola* developed characteristic symptoms of white leaf spot, and angular leaf spot, respectively. Both fungi could be reisolated from infected leaves showing characteristic symptoms of the respective diseases, and conidia and conidiophores of the reisolated fungi were morphologically similar to those of original isolates.

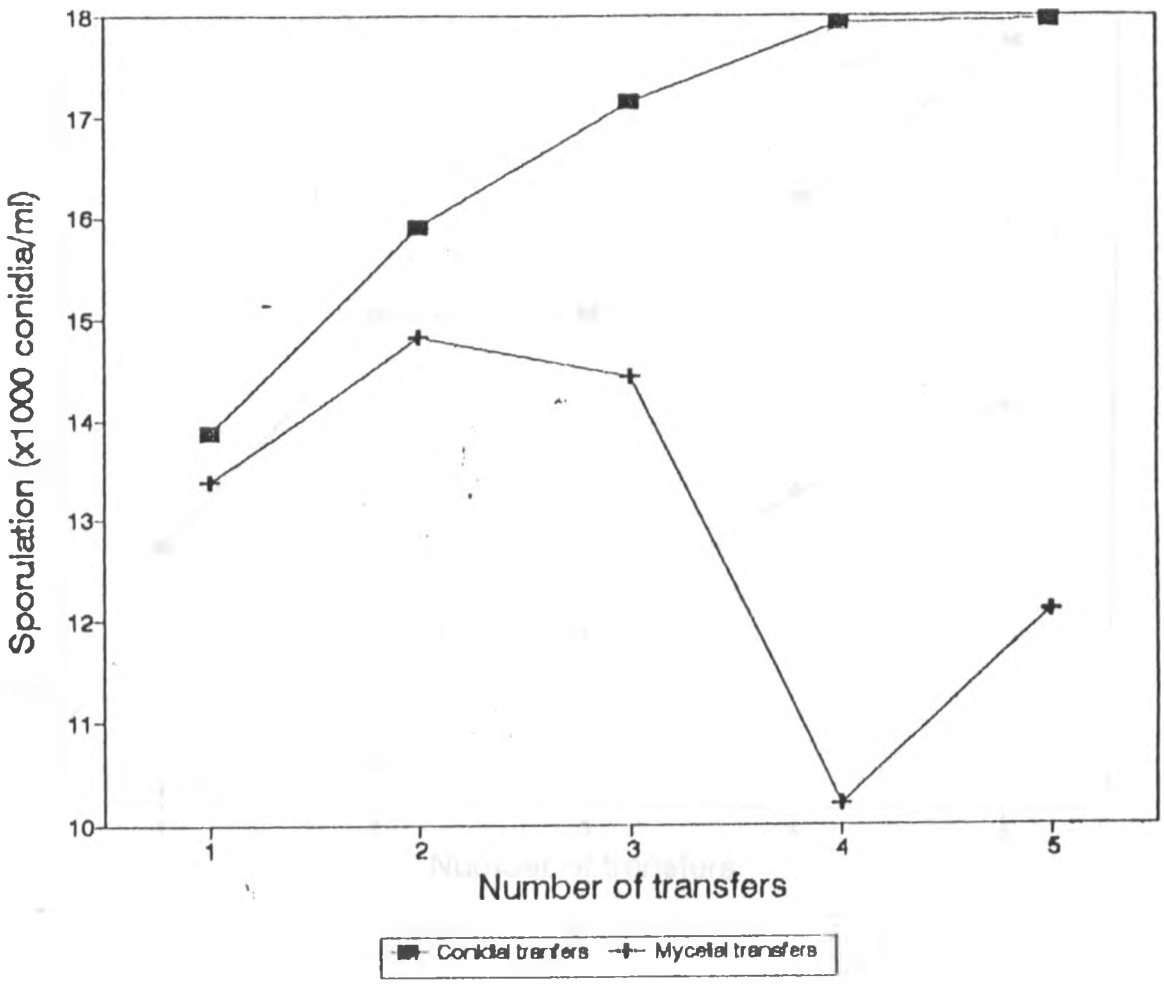

Fig. 11. Sporulation of *Cercospora sesami* on sesame stem meal agar 21 days after inoculation as influenced by interaction of inoculation technique and subculturing. (mean separation in table 7).

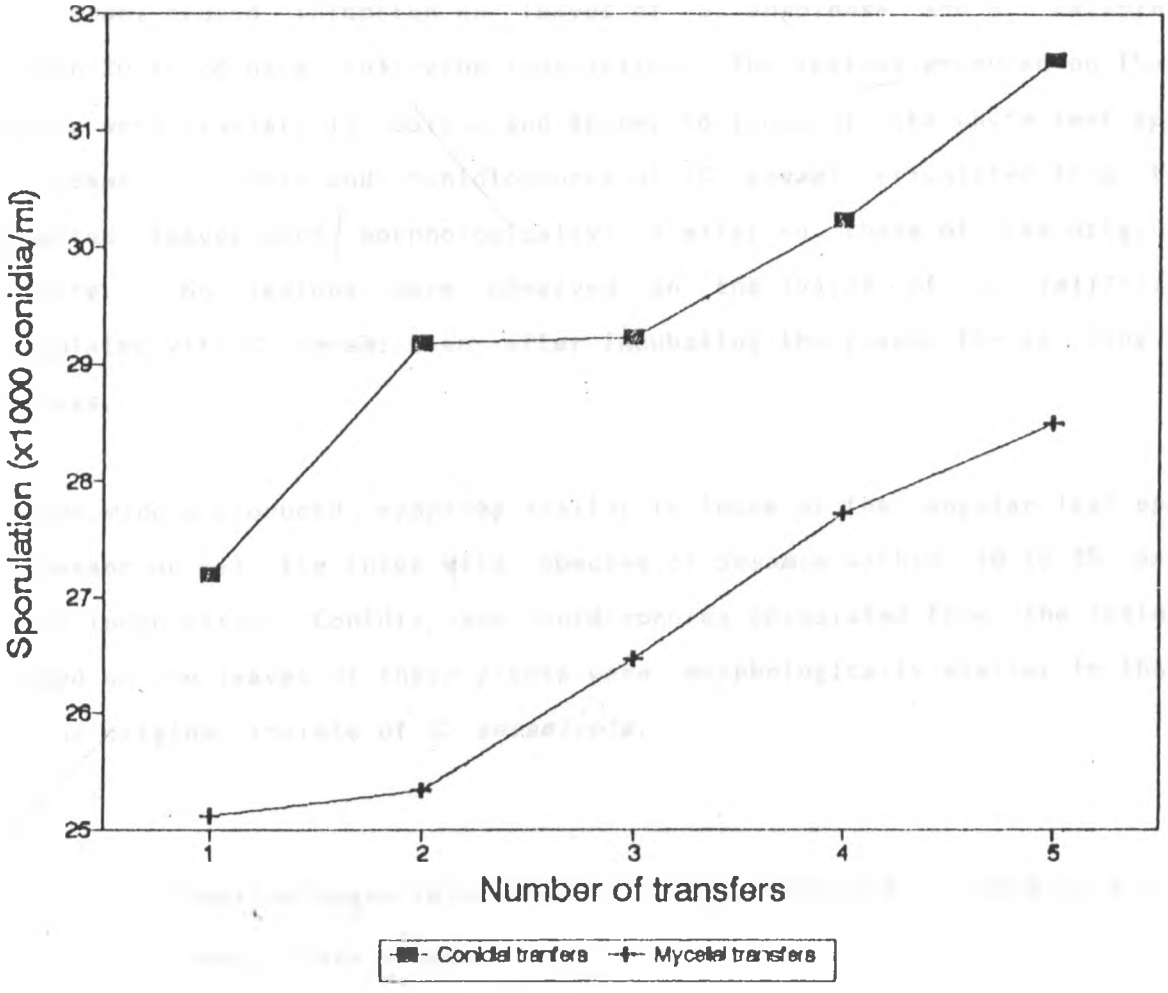

Fig. 12. Effect of inoculation technique and subculturing on sporulation of *Cercospora sesamicola* on sesame stem meal agar 21 days after inoculation. (mean separation in table 8).

4.2.1.2 Host range of *C. sesami* and *C. sesamicola*

C. sesami caused infection on leaves of *S. angolense* and *S. calycinum* within 20 to 28 days following inoculation. The lesions produced on these plants were similar, in colour and shape, to those of the white leaf spot of sesame. Conidia and conidiophores of *C. sesami* reisolated from the infected leaves were morphologically similar to those of the original isolate. No lesions were observed on the leaves of *S. latifolium* inoculated with *C. sesami* even after incubating the plants for as long as 42 days.

C. sesamicola produced symptoms similar to those of the angular leaf spot of sesame on all the three wild species of *Sesamum* within 18 to 35 days after inoculation. Conidia and conidiophores reisolated from the lesions formed on the leaves of these plants were morphologically similar to those of the original isolate of *C. sesamicola*.

4.2.2 Host-pathogen relationships of *C. sesami* and *C. sesamicola* on susceptible sesame

4.2.2.1 Pre-penetration and penetration events

Conidia of *C. sesami* germinated on inoculated leaf surface 3 to 6-hrs after inoculation to give 2 to 6 germtubes which measured 20 to 150u in length. Germtubes could arise from any of the conidial cells but the basal cells were always germinated first. No appressoria were observed but infection hyphae branched repeatedly prior to penetration. Penetration occurred 12 to 24-hrs after inoculation and was via the stomata. Although some germtubes were observed growing across the stomata, there was no evidence of direct penetration through intact plant surface (Plate 4).

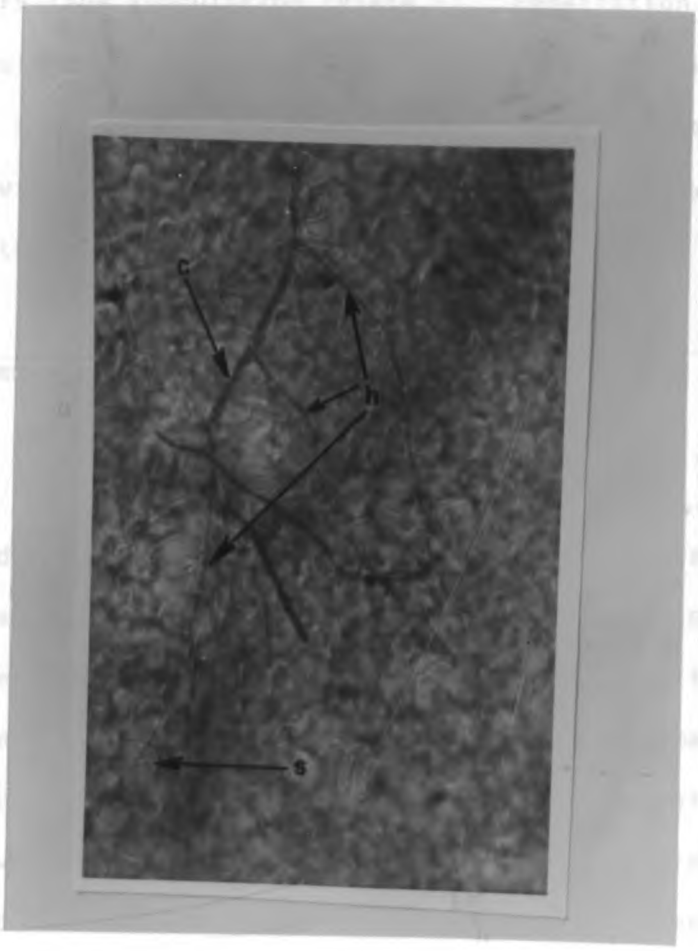

Plate 4. A germinating conidium (c) of *Cercospora sesami* on sesame leaf surface showing an infection hyphae (h) growing over the stomatal opening (s). Photograph taken 48-hrs after inoculation. Magnification = x 620

Conidial germination of *C. sesamicola* occurred 6 to 12-hrs after inoculation to give 1 to 3 very short germ tubes of 2 to 6u in length. Germtubes developed from any cell of the conidium and produced appressoria within 36 to 48-hrs from inoculation (Plate 5). Penetration occurred 48 to 60-hrs from inoculation, although considerable difficulty was experienced in observing this event; an examination of more than 200 germinated conidia revealed only 3 with infection hyphae that had gained entry into the leaf tissues. In all these cases, ingress was via the stomata.

4.2.2.2 Symptomatology

(a) White leaf spot of sesame

C. sesami attacked all aerial plant parts except the flowers. Macroscopic symptoms were first observed on the upper leaf surface 4 to 8 days after inoculation. These consisted of minute yellowish brown to light brown spots of less than 0.5-mm in diameter. The flecks later changed to become round purple coloured lesions and their size increased to attain diameters of 0.5 to 1.0-mm within 8 to 15 days from inoculation (Plate 6). After 20 to 28 days of incubation, the lesions had enlarged to reach diameters of up to 5-mm (range 3 to 5-mm), and their colour became white with raised purple to dark brown margins (Plate 6). Many of the infected leaves detached without much changes in lesion size or colour by the 42nd day of inoculation. By this time, stems and capsules also showed symptoms of infection. Lesions produced on the stem were purple with white centres and measured 2 to 5-mm in length; on capsules, the lesions were also purple with white centres and their diameter ranged from 1 to 3-mm (Plate 6)

(b) Angular leaf spot of sesame

C. sesamicola produced visible symptoms of infection only on the leaves and stems. The first symptoms appeared on the upper leaf surface 3 to 7 days from inoculation as minute chlorotic areas of less than 0.5-mm in diameter.

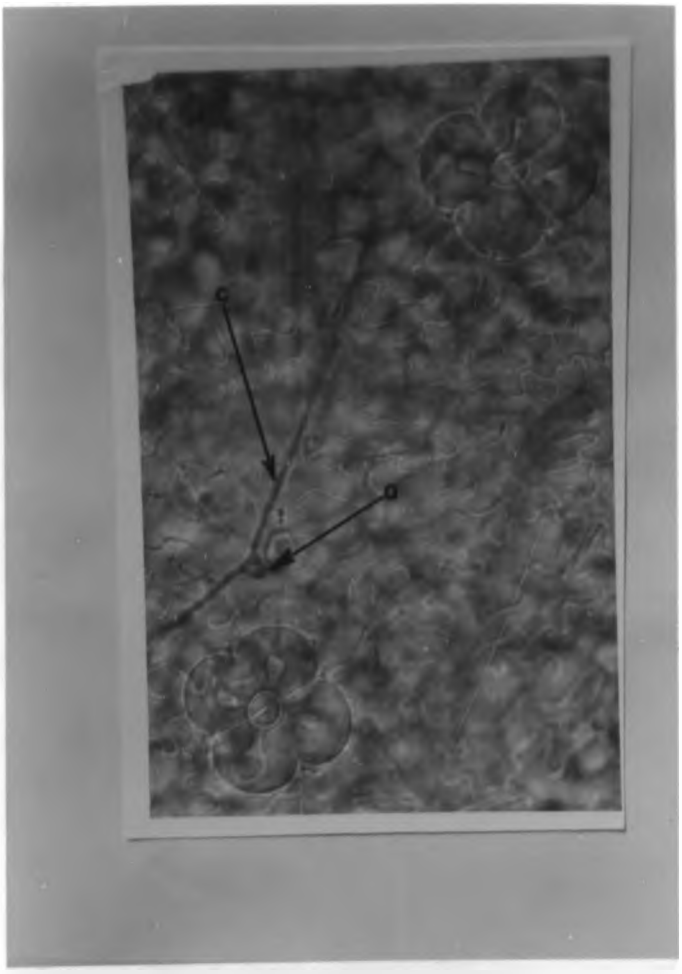

Plate 5. A germinating conidium (c) of *Cercospora sesamicola* on sesame leaf surface showing an appressorium (a) formation. Photograph taken 18- hrs after inoculation. Magnification = x 840

Plate 6. Stages of white leaf spot symptom development on susceptible sesame (Accession SIK 134). A) small round purple coloured lesions (pl). B) characteristic white centred spots with raised purple coloured margins (wpl). C) infected stem showing defoliation (df), stem lesions (sl) and capsule lesions (cl).

Three to five days later, the colour of the lesions changed to light brown and their shape became angular (Plate 7). Lesion colour on the upper leaf surface transformed to dark brown 24 to 35 days after inoculation; on the corresponding lower surface, the spots remained olivaceous brown but their margins became dark brown in colour. By this time, the size of the lesions ranged from 5 to 10-mm. Most of the infected leaves abscised by the 42nd day after inoculation. The first symptoms on the stem were first observed 28 to 35 days after inoculation. These comprised of tiny light brown spots of less than 0.5-mm in size. Within 42 days from inoculation, the lesions had elongated along the stem to attain lengths of 6 to 15-mm and their colour became olivaceous brown with light grey-silvery centres (Plate 7).

Plate 7. Stages of angular leaf spot symptom development on susceptible sesame (Accession SIK 134). A) small dark brown lesions with chlorotic margins (cl). B) characteristic dark brown angular spots (as) restricted by leaf veins. C) infected stem showing defoliation (df), stem lesions (sl).

4.3 Field experiments: Reaction of 16 sesame accessions to white and angular leaf spots

4.3.1 Reaction of 16 sesame accessions to white leaf spot at Siaya FTC and Kibwezi DRFS

4.3.1.1 Area under disease progress curves

Area under disease progress curves for percent diseased leaves (AUDPC-DL) were significantly larger at Kibwezi DRFS than at Siaya FTC ($t=2.565$, $p=0.05$). Highly significant differences in AUDPC-DL were also observed among the 16 sesame accessions at both experimental locations (Appendix 7). Accessions SPS 071 and SIK 134 exhibited the largest AUDPC-DL at both experimental locations (Table 9). The other accessions studied had significantly smaller AUDPC-DL except accessions SIK 005 and SPS 113 at Kibwezi DRFS, and accessions SIK 005, SIK 093 and SPS 113 at Siaya FTC. Judged by AUDPC-DL, disease was least severe on accession SPS 013 at both Kibwezi DRFS and Siaya FTC.

Like AUDPC-DL, area under disease progress curves for percent defoliation (AUDPC-DF) due to white leaf spot were also significantly larger at Kibwezi DRFS than at Siaya FTC ($t=5.945$, $p=0.01$). There were also highly significant differences in AUDPC-DF among the 16 accessions at both experimental locations (Appendix 7). Accession SPS 071 exhibited a significantly larger AUDPC-DF than did the other accessions tested at both Kibwezi DRFS and Siaya FTC (Table 9). Smallest AUDPC-DF was observed on accession SPS 031 at both experimental locations.

Table 9. Mean area under disease progress curves[a] for percent diseased leaves (AUDPC-DL) and percent defoliation (AUDPC-DF) from field tests conducted on 16 sesame accessions at Kibwezi DRFS FTC and Siaya FTC in October, 1991-February, 1992 to measure progress of white leaf spot

	Experimental location			
	Kibwezi DRFS		Siaya FTC	
Accession	AUDPC-DL	AUDPC-DF	AUDPC-DL	AUDPC-DF
SIK 005	3615.97ab	1369.67bc	3314.97abcd	1263.73bc
SIK 013	2357.13h	945.93defg	2173.97g	504.00e
SIK 015	2603.77gh	1161.07cde	2469.83efg	1290.10bc
SIK 031	2755.90efg	663.83g	2463.06efg	452.43e
SIK 093	3461.27bc	1428.93bc	3345.77abc	1200.73bc
SIK 122	3026.10de	873.37efg	2994.60bcde	662.90e
SIK 132	2866.26defg	1005.43def	2927.87bcde	773.50de
SIK 134	3860.50a	1161.07cde	3587.03a	1148.46bc
SPS 005	2884.70defg	1155.46cde	2854.37bcde	1050.23cd
SPS 007	2639.00fgh	766.50fg	2682.17efg	654.50e
SPS 028	2877.93defg	1271.43bcd	2777.60cdef	1097.37cd
SPS 045	3009.53def	1507.57b	2839.90bcde	1254.87bc
SPS 071	3868.43a	2311.63a	3867.967a	1981.00a
SPS 089	3225.04cd	1114.19cde	2236.96fg	780.50de
SPS 096	2649.50efgh	1585.03b	2736.30defg	1459.03b
SPS 113	3753.63ab	1355.90bc	3408.53ab	1080.57cd
Mean	3090.92	1256.34	2917.56	1040.87

[a] Average of 3 replications; within each experimental location, means followed by the same letter do not differ significantly at p=0.01 (Duncan's multiple range test)

4.3.1.2 Apparent rates of disease increase

The Gompertz model generally produced slightly higher coefficients of determination (R^2) than did the logistic model. Rates of increase in white leaf spot were, therefore, estimated and compared using the Gompertz model. Goodness of fit of the models to disease progress data, however appeared to vary from one sesame accession to another. The data in table 10 show the mean rates of disease increase on the 16 sesame accessions at either experimental site as was computed using the Gompertz model.

Rates of increase in percent diseased leaves (infection rates) due to white leaf spot were statistically similar at Kibwezi DRFS and Siaya FTC ($t=1.692$, $p=0.05$). There were, however, highly significant differences in infection rates among the 16 sesame accessions at either experimental site (Appendix 8). Maximum infection rate was observed on accession SPS 071 at Kibwezi DRFS. The rate of increase in percent diseased leaves was significantly slower on the other accessions studied except on SIK 005, SIK 093, SIK 134 SPS 005, and SPS 089. At Siaya FTC, percent diseased leaves increased most rapidly on accession SIK 093, but infection rate on this accession did not differ significantly from that on many of the other accessions tested (Table 10). Although the least rate of disease increase was observed on accession SIK 007 and SIK 015 at Kibwezi DRFS, infection rates on these accessions did not differ significantly from that on the other accessions studied apart from SIK 005, SIK 093, SIK 134, SPS 005, SPS 071 and SPS 089. At Siaya FTC, percent diseased leaves increased most slowly on accession SPS 007. However, infection rate on this accession did not differ significantly from that on the other accessions studied apart from SIK 093, SPS 071, and SIK 113.

Rates of increase in percent defoliation due to white leaf spot were significantly faster at Kibwezi DRFS than at Siaya FTC ($t=3.066$, $p=0.01$)

Table 10. Rates of increase [a] in percent diseased leaves and percent defoliation on 16 sesame accessions due to occurrence of white leaf spot at Kibwezi DRFS and Siaya FTC in October, 1991-February, 1992

	Experimental location			
	Kibwezi DRFS		Siaya FTC	
Accession	Infection rate	Defoliation rate	Infection rate	Defoliation rate
SIK 005	0.066ab	0.018bc	0.047abcd	0.019b
SIK 013	0.032f	0.017bc	0.032cd	0.015bc
SIK 015	0.032f	0.025bc	0.032cd	0.015bc
SIK 031	0.036ef	0.015c	0.033bcd	0.016bc
SIK 093	0.062abc	0.028bc	0.067a	0.021ab
SIK 122	0.044def	0.016bc	0.047abcd	0.011c
SIK 132	0.037ef	0.017bc	0.048abcd	0.015bc
SIK 134	0.057abcd	0.019bc	0.048abcd	0.014bc
SPS 005	0.051bcde	0.024bc	0.043abcd	0.018bc
SPS 007	0.034ef	0.022bc	0.024d	0.014bc
SPS 028	0.043def	0.019bc	0.038bcd	0.015bc
SPS 045	0.043def	0.025bc	0.041bcd	0.019b
SPS 071	0.069a	0.065a	0.052abc	0.027a
SPS 089	0.059abcd	0.029b	0.030cd	0.014bc
SPS 096	0.038ef	0.022bc	0.039bcd	0.018bc
SPS 113	0.048cdef	0.020bc	0.059ab	0.017bc
Mean	0.048	0.0378	0.0425	0.0186

[a] Rates of increase were obtained by regressing Gompertz-transformed disease against time (expressed as days after planting).

[b] Values represent average of 3 replications; within each experimental location, means followed by the same letter do not differ significantly at p=0.01 (Duncan's multiple range test)

Differences in defoliation rates among the 16 sesame accessions at either experimental location were, however, highly significant (Appendix 8). At both Kibwezi DRFS and Siaya FTC. accession SPS 071 showed a significantly faster rate of defoliation than did the other accessions tested except SIK 093 at Kibwezi DRFS. Although the slowest rate of increase in percent defoliation was observed on accession SIK 031 at Kibwezi DRFS, defoliation rate on this accession did not differ significantly from that on the other accessions tested apart from SPS 071 and SPS 089. At Siaya FTC, defoliation rate was slowest on accession SIK 122. However, the rate of defoliation on this accession did not differ significantly from that on the other accessions tested apart from SIK 005, SIK 093, SPS 045, and SPS 071.

4.3.2 Reaction of 16 sesame accessions to angular leaf spot at Siaya FTC and Kibwezi DRFS

4.3.2.1 Area under disease progress curves

Area under diseasa progress curves for percent diseased leaves (AUDPC-DL) were significantly larger at Kibwezi DRFS than at Siaya FTC ($t=2.76$, $df=15$, $p=0.01$). There were also significant differences in AUDPC-DL among the 16 sesame accessions at both experimental locations (Appendix 9). Accessions SIK 122, SIK 132, and SIK 134 exhibited the largest AUDPC-DL at Kibwezi DRFS (Table 11). The other accessions studied had significantly smaller AUDPC-DL except SPS 015, SPS 071, and SPS 028. At Siaya FTC, accessions SIK 132 and SIK 134 had significantly larger AUDPC-DL than that on all the other accessions studied. Judged by AUDPC-DL, least disease severity was observed on accessions SIK 031, SPS 045 and SPS 096 at Kibwezi DRFS. Angular leaf spot severity was significantly greater on the other accessions tested except on SPS 005, SPS 089, and SPS 113. At Siaya FTC, smallest AUDPC-DL was exhibited by accessions SIK 031 and SPS 045.

Table 11. Mean area under disease[a] progress curves for percent diseased leaves (AUDPC-DL) and percent defoliation (AUDPC-DF) from field tests conducted on 16 sesame accessions at Siaya FTC and Kibwezi DRFS in Oct., 1991-Feb., 1992 to measure progress of angular leaf spot

	Experimental location			
	Kibwezi DRFS		Siaya FTC	
Accession	AUDPC-DL	AUDPC-DF	AUDPC-DL	AUDPC-DF
SIK 005	3719.10bc	767.67fg	3454.73bcde	760.90b
SIK 013	3687.13bc	1365.00d	3496.50bc	246.87d
SIK 015	3978.10ab	890.40ef	3153.73bcde	1027.37a
SIK 031	3027.97e	217.93j	2514.10h	222.60d
SIK 093	3721.20bc	805.00f	3432.57bcd	185.97d
SIK 122	4344.60a	491.63hi	3087.93cdef	260.63d
SIK 132	4275.367a	2578.33a	4529.70a	1068.20a
SIK 134	4269.30a	1891.63b	4419.10a	500.50c
SPS 005	3421.37cde	600.13gh	3327.33bcde	518.47c
SPS 007	3630.67bcd	540.17hi	3011.63defg	344.40d
SPS 028	3867.97ab	1008.47e	2966.60efg	790.96b
SPS 045	3086.77e	393.631j	2459.57h	276.97d
SPS 071	3890.37ab	1596.23c	3563.23b	511.70c
SPS 089	3269.23cde	805.00f	2671.67fgh	756.00b
SPS 096	3107.07e	916.77ef	2958.20efg	504.67c
SPS 113	3221.87de	489.77hi	2579.27gh	794.73b
Mean	3594.88	965.58	3226.64	518.77

[a] Average of 3 replications; within each experimental location, means followed by the same letter do not differ significantly at p=0.01 Duncan's multiple range test)

The other accessions evaluated had significantly larger AUDPC-DL except SPS 089 and SPS 113.

Like AUDPC-DL, AUDPC-DF due to angular leaf spot were also significantly larger at Kibwezi DRFS than at Siaya FTC ($t=2.907$, $df=15$, $p=0.01$). Differences in AUDPC-DF were also highly significant among the 16 sesame accessions at both experimental sites (Appendix 3.5.2a). Accession SIK 132 exhibited significantly larger AUDPC-DF than did all the other accessions studied at Kibwezi DRFS (Table 11). At Siaya FTC, AUDPC-DF on accession SIK 132 was significantly larger than on all the other accessions tested with the exception of SIK 015. Smallest AUDPC-DF was observed on accession SIK 031 at Kibwezi DRFS. The other accessions had significantly greater AUDPC-DF except SPS 045. At Siaya FTC, AUDPC-DF on accession SIK 031 was not only similar to that on accession SPS 045, but also to that on the accessions SIK 013, SIK 093, SIK 122, and SPS 007. The other accessions tested had significantly larger AUDPC-DF.

4.3.2.2 Apparent rates of disease progress

As was the case with white leaf spot, the Gompertz model produced slightly higher coefficients of determination (R^2) than did the logistic model for angular leaf spot severity data. Goodness of fit of the model for disease progress data appeared to vary from one sesame accession to another. The data in table 12 summarise the mean rates of disease increase on the 16 sesame accessions at the two experimental sites as was estimated using the Gompertz model.

Rates of increase in percent diseased leaves due to angular leaf spot were significantly greater at Kibwezi DRFS than at Siaya FTC ($t=2.133$, $df=15$, $p=0.05$). Differences in infection rates among the sesame accessions were not significant at Kibwezi DRFS, unlike at Siaya FTC (Appendix 10).

Table 12. Rates of increase[a] in percent diseased leaves and percent defoliation on 16 sesame accessions due to occurrence of angular leaf spot at Kibwezi DRFS and Siaya FTC in Oct.,1991-Feb., 1992

	Experimental location			
	Kibwezi DRFS		Siaya FTC	
Accession	Infection rate	Defoliation rate	Infection rate	Defoliation rate
SIK 005	0.053abcd	0.015bcd	0.061ab	0.012cde
SIK 013	0.051abcd	0.016abc	0.029cd	0.014cd
SIK 015	0.059ab	0.017ab	0.019d	0.009de
SIK 031	0.034cd	0.013bcde	0.041abcd	0.011cde
SIK 093	0.040abcd	0.015bcd	0.028cd	0.013cd
SIK 122	0.035bcd	0.008e	0.023cd	0.007def
SIK 132	0.056abc	0.016abc	0.062ab	0.025a
SIK 134	0.053abcd	0.013bcde	0.066a	0.016abc
SPS 005	0.053abcd	0.011cde	0.036bcd	0.012cde
SPS 007	0.052abcd	0.010de	0.030cd	0.002f
SPS 028	0.047abcd	0.010de	0.050abc	0.007def
SPS 045	0.031d	0.017d	0.013bcde	0.010cde
SPS 071	0.061a	0.020a	0.066a	0.023ab
SPS 089	0.044abcd	0.012bcde	0.031cd	0.005ef
SPS 096	0.040abcd	0.013bcde	0.043abcd	0.011cde
SPS 113	0.062a	0.012bcde	0.044abcd	0.010cde
Mean	0.048	0.013	0.040	0.012

[a] Rates of increase were obtained by regressing Gompertz-transformed disease against time (expressed as days after planting); values shown represent average of 3 replications; within each experimental location, means followed by the same letter do not differ significantly at p=0.01 (Duncan's multiple range test)

Although the most rapid rate of increase in percent diseased leaves was observed on accessions SPS 071 and SPS 113 at Kibwezi DRFS, infection rate on these accessions did not differ significantly from that on the other accessions tested except SIK 031, SIK 122 and SPS 045. At Siaya FTC, percent diseased leaves increased most rapidly on accessions SIK 134 and SPS 071. Infection rates were significantly slower on the other accessions studied except on SIK 005, SIK 031, SIK 132, SPS 028, SPS 096 and SPS 113. Least rate of increase in percent diseased leaves rates was exhibited by accession SPS 045 at both experimental sites. However, infection rate on this accession did not differ significantly from that on the other accessions studied except on SIK 015, SIK 132, SPS 028SPS 071 and SPS 113, and on SIK 005, SIK 132, SIK 134, SPS 028 and SPS 071 at Kibwezi DRFS, and Siaya FTC, respectively.

Rates of increase in percent defoliation due to angular leaf spot at the two experimental sites were not significantly different ($t=1.087$, $p=0.05$). However, there were significant differences in defoliation rates among the 16 sesame accessions at both experimental locations (Appendix 10). The fastest defoliation rate was exhibited by accession SPS 071, and SIK 132 at Kibwezi DRFS, and Siaya FTC, respectively. Although the slowest rate of increase in percent defoliation was observed on accession SIK 122 at Kibwezi DRFS, defoliation rate on this accession did not differ significantly from that on most of the other accessions tested (Table 12). At Siaya FTC, slowest rate of increase in defoliation was exhibited by accession SPS 007. The other accessions studied had significantly faster rates of defoliation except SIK 122, SPS 028, and SPS 089.

CHAPTER 5

DISCUSSIONS

5.1 Colony characteristics of *C. sesami* and *C.sesamicola*

Although colonies of *C. sesami* were larger than those of *C. sesamicola* on all solid media, mycelial dry weights of the two fungi were similar when they were cultured on liquid media. This may be explained by tendency for *C. sesamicola* cultures to grow upwards rather than laterally, unlike *C. sesami*. Due to this, colony diameter may not be a reliable parameter for comparing *in vitro* growth of the two fungi. Lilly and Barnett (1951) also discourage the use of colony diameter when precise measurement of *in vitro* fungal growth is desired. Colony characteristics of the two *Cercosporae* on solid media may, however, be useful for their differentiation. Sobers (1969) also recognized the potential for using colony morphology in distinguishing species of *Cercospora*.

5.2 Morphological characteristics of *C. sesami* and *C.sesamicola*

Although *C. sesami* and *C. sesamicola* have previously been differentiated by their *in situ* morphological features, this study shows that conidia and conidiophores produced by these fungi on SSMA may also aid in their identification. *C. sesami* produced larger conidia and conidiophores than *C. sesamicola*. Conidial septation and shape were also markedly different between the two fungi. These findings compare well with descriptions of the two fungi by Mohanty (1958), and Ellis (1971). The slight differences in the conidial and conidiophore dimensions observed in this work could be due to effects of the artificial conditions under which the two fungi were cultured. Berger and Hanson (1963b) also observed variation in sizes of conidia and conidiophores of several *Cercospora* species following their production under artificial conditions.

5.3 Cultural conditions influencing growth and sporulation of C. sesami and *C. sesamicola*

Cultures of *C. sesami* and *C. sesamicola* grew and sporulated over the entire range of conditions investigated. However, optimal mycelial growth and conidial production occurred only under some specific conditions.

Media composition and quantity influenced growth and sporulation of both fungi, but either factor exerted its effects independently. The profuse growth of both fungi on media containing potato dextrose and in plates carrying large quantities of media (35-ml per 9-cm diameter plate) could be due to the greater availability of utilisable carbon in the two cases (Verma and Agnihotri, 1972). Berger and Hanson (1963) also observed that increased growth of *Cercospora zebrina* cultures with increased concentration of glucose in culture media. Unlike growth, conidial production by *C. sesami* was greatest in plates containing small quantities of medium (15-ml), and sesame stem meal agar (SSMA) medium. This was, possibly, as a result of the faster rate of nutrient depletion in such plates (Ekpo and Esuruoso, 1978). Chowdhury (1944) also observed maximum sporulation of *C. sesami* on media prepared from sesame stems. Although Kilpatrick and Johnson (1956) reported sporulation of *C. sesami* on carrot leaf decoction agar (CLDA), only moderate quantities of conidia were produced on this media. This study showed that sporulation of *C. sesami* is markedly better on SSMA than on CLDA. Maximum sporulation of *C. sesamicola* also occurred on SSMA, but in contrast to *C. sesami*, spores of this fungus were most abundant in plates carrying large quantities of media (35-ml). This is because the effect of media quantity on sporulation of this fungus on most media followed a pattern similar to that of its growth, possibly as a result of the direction of mycelial production towards the formation of sporulating structures and their supporting hyphae, rather than vegetative structures (Calpouzos, 1954).

Effect of pH on *in vitro* growth and sporulation of both fungi was dependent on the duration of incubation. This could be as a result of alteration of

media pH by the two fungi during growth. Other workers have also reported change of media pH by cultures of *Cercospora* species. (Berger and Hanson, 1963; Verma and Agnihotri, 1972). Although *C. sesami* and *C. sesamicola* grew over a wide range of pH, optimum pH for growth was between pH 6 and pH 7 for all the durations of incubation tested. This compares well with the findings of Chowdhury (1944) who observed optimum growth of *C. sesami* at pH 6.5. Some workers have also observed optimum growth of other *Cercospora* species between pH 6 and 7 (Rangaswami and Chandraskeran, 1962; and Verma and Agnihotri, 1972). The effect of incubation period on sporulation of both fungi generally followed a pattern similar to that of their growth at most of the tested media pH. The decline in conidial production by *C. sesami* after the 21st day of incubation was due to germination of some conidia to give vegetative hyphae. Cooperman and Jenkins (1986) also observed conidial disintegration through germination following prolonged incubation of cultures of *C. asparagi*. Although conidia of *C. sesamicola* also germinated following prolonged incubation, secondary conidia rather than vegetative hyphae were formed in the process. This may explain the consistency in sporulation of this fungus with prolonged incubation. Berger and Hanson (1963a) also observed formation of secondary conidia, following germination of spores of *C. zebrina*.

Growth and sporulation of *C. sesami* and *C. sesamicola* were also affected by interaction of light and temperature. Although this interaction is known to influence sporulation of some other fungi such as *Cercospora beticola*, *Aschochyta pisi*, and *Alternaria tomato* (Aragaki, 1961; Leach, 1962; Calpouzos and Stallknecht, 1965), its fundamental nature is not well understood. However, the effect of temperature on growth of *C. sesami* and *C. sesamicola* under each of the three light regimes followed a pattern similar to that of other *Cercospora* species (Berger and Hanson, 1963a; Verma and Agnihotri, 1972; Ekpo and Esuruoso, 1978; Chen *et al.*,1979; Cooperman and Jenkins, 1986). It was suggested that this pattern resembles that of an enzyme reaction, with growth increasing to an optimum at about 25^0 C, then dropping to zero at very high temperatures (above 35^0 C). Sporulation of both *C. sesami* and *C. sesamicola* occurred under continuous

darkness at all the temperatures investigated in this study; therefore, no particular wavelength of light is apparently necessary for spore production. This does not seem to be true for some *Cercospora* species such as *C. beticola* and *C. kikuchii* whose spore initiation are thought to require light (Calpouzos and Stallknecht, 1965; Chen *et al.*, 1979). It agrees, however, with the findings of Cooperman and Jenkins (1986) who also observed abundant sporulation of *C. asparagi* under continuous darkness. As conidia of *C. sesami* were most abundant under alternating light/dark cycles and least plentiful under continuous light, a dark period following illumination seems to stimulate sporulation of this fungus. This does not appear to be the case with *C. sesamicola* whose sporulation was greatest under continuous light.

The increase in growth of *C. sesami* and *C.sesamicola* with successive subculturing may have been as a result of nutritional adaptation of these fungi to the culture medium (Lilly and Barnett, 1951). However, the corresponding rise in sporulation of *C. sesami* associated with repeated transfer of cultures produced from mycelial fragments could have been as a result of selective subculturing of sporulating sectors of the colonies, and vice versa (Nagel, 1934; Jones, 1958; Calpouzos and Stallknecht, 1965). This may also explain why there was an improvement in conidial production by the same fungus in cultures that were produced from conidia. In *C. sesamicola*, subculturing improved sporulation even when cultures were obtained from mycelia, possibly because most of its mycelial growth was directed towards formation of sporulating structures and their supporting hyphae; for this reason, the mycelial transfers also contained some conidia. Colonies of the two fungi were larger when propagated through transfers of mycelial fragments rather than single conidia, possibly because the former inoculation technique involved use of a larger amount of inoculum. Fungal growth of fungi is generally more profuse with heavy inoculum rather than light inoculum (Cochrane, 1958). Sporulation of both *C. sesami* and *C. sesamicola* was, however, more abundant with transfers of single conidia rather than mycelial fragments. This compares well with the findings of many workers who have generally been unsuccessful in obtaining

conidia of *Cercospora* species from cultures prepared using mycelium (Nagel, 1938; Kilpatrick and Johnson, 1958; Ekpo and Esurudso, 1978).

5.4 Pathogenicity of *C. sesami* and *C. sesamicola* on sesame and on wild species of *Sesamum*

Although sesame is listed as the natural host for *C. sesami* and *C. sesamicola* (Mohanty, 1958; Ellis, 1971), there is no evidence that the 3rd and 4th Koch's postulates were tested to provide proof for pathogenicity of the two fungi on this crop. This study confirms that *C. sesami*, and *C. sesamicola* are not merely associated with sesame but are indeed the causal agents of the white leaf spot, and angular leaf spot, respectively.

Apart from infecting sesame, *C. sesami* has also been observed on a related species of the crop, *S. angolense*, in Kenya (Nattrass, 1961). However, no studies were conducted to determine whether the *C. sesami* observed on either host were identical in their pathogenicity. This study showed that *C. sesami* isolated from sesame is also pathogenic to *S. angolense*. The same isolate also caused infection on *S. calycinum*, but not *S. latifolium*. In contrast, the *C. sesamicola* isolate from sesame infected all the three wild species of *Sesamum*. Some *Cercosporae* are known to infect plant species related to those from which they were isolated (Baxter, 1956; Latch and Hanson, 1962; Berger and Hanson, 1963b; Pons ,1988), but others cause infection only on their natural hosts (Jones, 1944).

The ability of *C. sesami* and *C. sesamicola* to infect the specified wild species of *Sesamum* is an indication that these plants may act as alternative hosts to the two pathogens (Roy, 1988). The plants should, therefore, be eradicated from the neighbourhood of sesame fields if control of the white and angular leaf spots of sesame through measures such as seed treatment are to be effective. Eradication of alternative hosts is recommended for control of many fungal diseases of plants such as club root of crucifers caused by *Plasmodiophora brassicae*, and take-all disease of

wheat caused by *Gaeumannomyces graminis* var *tritici* (Manners, 1982; Jones, 1987)

The inability of *C. sesamicola* to infect *S. latifolium* implies that this species may be useful as a source of resistance to the angular leaf spot of sesame. Wild relatives of several crops have been found to exhibit resistance to many fungal diseases (Knott and Dvorak, 1976; Bowder, 1980; Tomerlin, 1989)

5.5 Host-pathogen relationships of *C. sesami* and *C. sesamicola* on susceptible sesame

Adequate knowledge of histopathological relationships of plant diseases is prerequisite for furthering knowledge of parasitism. Observations made on germination and penetration of *C. sesami* and *C. sesamicola* on the leaf surface of susceptible sesame in this study, are in agreement for the most part, with the findings of many other workers who have investigated host-pathogen systems of many *Cercospora* leaf spots (Jenkins, 1938; Baxter, 1956, Berger and Hanson, 1963b; Abdou *et al.*, 1974). Although the formation of appressoria by *C. sesamicola* seems to be uncharacteristic of *Cercospora* species, the same phenomena has been reported for *C. beticola* (Solel and Minz, 1971), *C. arachidicola* (Melouk and Aboshosha, 1989), *C. zeae maydis* (Thorson and Martison, 1989).

Observations made on the stages of development of white, and angular leaf spot symptoms on susceptible sesame are comparable with descriptions of the two diseases by Mohanty (1958), Schiller *et al.* (1981), and Kolte (1985).

5.6 Reaction of sesame accessions to white and angular leaf spots

Although some workers have investigated the susceptibility of different genotypes to the two *Cercospora* leaf spots of sesame (Kushwasha and Kaushal, 1970; Singh *et al.*, 1976; Vyas, 1981; Rao and Dhamu, 1983;

Kurozawa *et al.*, 1985), no information was available on the relative susceptibility of the sesame genotypes found in Kenya. This study showed that of all the accessions evaluated under conditions at Siaya FTC and Kibwezi DRFS, accessions SIK 031 and SPS 045, and accession SPS 013 were relatively less susceptible to the white leaf spot, and angular leaf spot, respectively. Therefore, these accessions may be useful as standards for determining the resistance of sesame selections to the respective *Cercospora* leaf diseases in future; selections found to have lower AUDPCs may be considered resistant.

The disease progress models used in this study did not fit disease data from all sesame accessions equally well. Although the Gompertz model provided statistically significant fits to disease progress data for all of the sesame accessions evaluated, coefficients of determination of Gompertz-transformed disease data varied across the accessions. This problem could have been avoided by using more mathematically explicit models such as the Wiebull model (Pennypecker, 1980), but such models are often too computationally complex to use when evaluating more than just a few genotypes.

Apart from making it possible for the avoidance of problems associated with imperfect fits of disease data and being relatively easy to calculate, AUDPCs appeared also to be better indicators of relative susceptibility of sesame accessions to both white leaf spot and angular leaf spot than estimates of infection or defoliation rate. Rates of disease increase were averaged over the entire season, while AUDPCs were computed from averages of disease data over 10 to 14 day intervals. AUDPCs may, therefore, reflect timing of disease increase more accurately than rates of disease increase. Johnson and co-workers (1986) also made similar observations while studying the early leaf spot of peanut (*Arachis hypogea*) caused by *C. sesamicola*.

5.7 Conclusions and recommendations

Development of varieties of sesame that are resistant to white, and angular leaf spot has been slow due to the lack of suitable techniques for creating uniform and consistent epiphytotics of these diseases. Inoculum necessary for the induction of such epiphytotics was inadequate since *C. sesami* and *C. sesamicola* produced only scarce quantities of conidia on culture media. This study has shown that to produce large quantities of conidia of both fungi, plates containing sesame stem meal agar medium at a pH of 6 to 7 should be inoculated using conidia obtained from the 4th or 5th subcultures. For maximum sporulation, inoculated plates should be incubated at 25 ^{0} C under 12-hr alternating dark/light for 14-21 days for cultures of *C. sesami*; for *C. sesamicola*, the plates should be incubated at 25 ^{0} C under continuous light for 21-28 days. The spores formed after the respective durations of incubation can be harvested by flooding colony surfaces with water and lightly stroking them with a small brush or glass rod.

It is worth noting, however, that information is still needed on the effect of subculturing on pathogenicity of both fungi before inocula from subcultures can be widely recommended for inducing epiphytotics of the specified diseases. This is because, even though subculturing appears to improve sporulation of the two fungi, it may also cause avirulence of pathogen, and hence, make it possible for spurious conclusions to be reached on the results of disease assessments made while using such inoculum.

Seed treatment and destruction of crop residues are currently the most widely adopted methods of controlling the *Cercospora* leaf diseases of sesame in Kenya. However, because this study demonstrated that *C. sesami* and *C. sesamicola* can also infect some wild relatives of sesame, these control measures may be rendered ineffective due to such plants acting as alternative sources of inoculum. The wild relatives of sesame known to be

alternative hosts of the two fungi should, therefore, be eradicated from the neighbourhood of sesame fields.

Research should be conducted to asses pathogenicity of the two *Cercosporae* on other wild relatives of sesame such as *S. angustifolium*, *S. radiatum* and *Ceratotheca sesamoides* as these may also be alternative hosts to the two fungi. More information is also needed on the relative susceptibility of the wild species of *Sesamum* to the two *Cercospora* species, since some of them could be useful sources of resistance to these fungi.

Comparative studies on the phases of the infection process of *Cercospora* species on susceptible and resistant hosts has played an important role in understanding mechanisms of resistance to diseases caused by these fungi. This study demonstrated germination and penetration of *C. sesami* and *C. sesamicola* on a susceptible sesame accession (SIK 134). Conidia of the two fungi germinated within 3 to 12-hrs from inoculation to give germtubes which later on produced appressoria in the case of *C. sesamicola*, but not in *C. sesami*. Penetration of infection hyphae of both fungi into leaf tissues occurred via the stomata 12 to 36-hrs after inoculation.

Similar studies should be conducted on sesame accessions with varied levels of reaction to the white and angular leaf spot diseases to determine whether such differences can be attributed to variations in the phases of the infection process. This may be useful in understanding mechanisms of resistance to the two diseases.

Although some sesame germplasm have been found to exhibit resistance to the two *Cercosporae* in other parts of the world, evaluation of such materials in Kenya may have been difficult previously due to the lack of standard genotypes with which they could be compared. The reaction of such materials in Kenya can presently be compared with that of accessions SIK 031 and SPS 045, and accession SPS 013 which, in this study, exhibited the least susceptibility to the white leaf spot, and angular leaf spot.

respectively.

More research should be conducted on these accessions to determine the stability as well as components of their reaction to the two *Cercospora* diseases. Studies should also be undertaken to develop a disease scoring scale for either *Cercospora* leaf spot of sesame so as to facilitate rapid screening of large amounts of sesame germplasm.

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7. APPENDICES

Appendix 1. Culture media and their composition

The term autoclaved refers to sterilised by steaming without pressure at 98^0 C for 1-hr

1.1 Carrot leaf decoction agar (CLDA)

Carrot leaves	200 g	Freshly picked leaves were finely ground in a blender. 500-ml of sterile distilled water water was added and followed by boiling for 30 minutes. The solution was strained through two layers of cheese cloth. volume adjusted to 1 l with sterile distilled water, agar added, and the ingredients autoclaved.
Agar	20 g	
Water	1000 ml	

1.2 Carrot leaf decoction potato dextrose agar (CLDPDA)

Carrot leaves	200 g	Carrot leaf decoction was prepared as described in 1.1 above. Oatmeal was also boiled in sterile distilled water for 30-minutes. The two solution were strained through two layers of cheese cloth. mixed and volume adjusted to 1 litre. Agar was added and the ingredients autoclaved.
Rolled oats	20 g	
Agar	20 g	
Water	1000 ml	

1.3 Corn meal agar (CMA)

Maize meal	30 g	Ingredients were heated to simmering and then autoclaved
Agar	20 g	
Water	1000 ml	

1.4 Malt extract agar (MEA)

Malt extract	20 g	Ingredients were heated to simmering and then autoclaved
Agar	20 g	
Water	1000 ml	

Cont.....Appendix 1

1.5 Oatmeal agar (OA)

Rolled oats	20 g	Oatmeal decoction was prepared as described
Agar	20 g	in 1.2. Volume was adjusted to 1 l, agar
Water	1000 ml	added, and the ingredients autoclaved.

1.6 Potato carrot agar (PCA)

Potato	20 g	Sliced potato and carrot tissue were heated
Carrot	20 g	to simmering and then boiled for 5 minutes.
Agar	20 g	Mixture was strained through two layers of
Water	1000 ml	cheese cloth, agar added and ingredients autoclaved

1.7 Potato dextrose agar (PDA)

Potato extract	4 g	Ingredients heated to simmering and then
Dextrose	20 g	autoclaved
Agar	20 g	
Water	1000 ml	

1.8 Sesame leaf decoction agar (SLDA)

Sesame leaves	200 g	Decoction was prepared from susceptible
Agar	20 g	leaves of 8-wk old susceptible sesame
Water	1000 ml	(accession SIK 134) as described for carrot leaf decoction in (i). Volume was adjusted to 1 l, agar added and ingredients autoclaved.

1.9 Sesame leaf decoction oatmeal agar (SLDOA)

Sesame leaves	200 g	Decoctions were prepared from sesame leaves
Rolled oats	20 g	and rolled oats as described in (ii). Volume
Agar	20 g	was adjusted to 1 l and ingredients autoclaved
Water	1000 ml	

cont.....Appendix 1

1.10 Sesame leaf decoction potato-dextrose agar (SLDPDA)

Sesame leaves	200 g	Sesame decoction was prepared as described in
Potato extract	4 g	for carrot leaf decoction in (i). Potato
Dextrose	20 g	extract, dextrose, and agar were added to the
Agar	20 g	decoction, and ingredients autoclaved
Water	1000 ml	

1.11 Sesame stem-meal agar (SSMA)

Sesame stems (dry)	5 g	Stems of mature susceptible sesame (accession
Agar	20 g	SIK 134) were dried in the oven at 60 for 12
Water	1000 ml	hours and ground to fine flour. Distilled water and agar were added and ingredients autoclaved

1.12 Sesame stem-meal oatmeal agar (SSMOA)

Sesame stems (dry)	5 g	Fine flour was prepared from sesame stems as
Rolled oats	20 g	as described in (xi). Oatmeal decoction
Agar	20 g	prepared as outlined in (iv) and agar were
Water	1000 ml	added and ingredients autoclaved

1.13 Sesame stem meal potato dextrose agar (SSMPDA)

Sesame stems (dry)	5 g	Fine flour made from sesame stem as described
Potato extract	4 g	in (xi) was mixed with the specified
Dextrose	20 g	components and the ingredients autoclaved.
Agar	20 g	
Water	1000 ml	

Appendix 2. Description of the experimental sites

2.1 Geographical location

	Kibwezi DRFS	Siaya FTC
Altitude	804 m	914 m
Latitude	2 ^{0} 17' 00'' S	0 ^{0} 3' 16'' N
Longitude	38 ^{0} 36' 36'' E	34 ^{0} 17' 43'' E

Source: Ekirapa and Muya (1991), Kadenge Agrometeorological Station.

2.2 Soils

	Kibwezi DRFS	Siaya FTC
Colour	Deep to dusky red	Dark reddish brown
Depth	Deep	Shallow
Drainage	Good	Moderate
Texture	Sandy clay to clay	Sandy loam
Name	eutric luvisol	dystric fluvisol

Source Ekirapa and Muya (1991), Kadenge Agrometeorological Station

cont.....Appendix 2

2.3 Meterological data for Oct., 1991-Feb., 1992

	Kibwezi DRFS					Siaya FTC				
	Oct.	Nov.	Dec.	Jan.	Feb	Oct.	Nov.	Dec.	Jan.	Feb.
Temperature (^{0} C)										
Min.	10.4	18.6	18.3	15.5	13.8	17.6	17.8	17.5	16.7	17.4
Max.	32.2	30.2	29.9	28.4	31.3	30.3	28.8	31.1	30.8	31.1
Mean	21.3	24.4	24.1	21.9	22.6	24.0	23.3	24.3	23.8	24.3
Humidity										
Min.	-	-	-	-	-	-	58.0	-	50.0	50.0
Max.	-	-	-	-	-	-	69.0	-	81.0	79.0
Mean	-	-	-	-	-	-	63.5	-	65.5	64.5
Rainfall (mm)	13.7	182.3	181.7	13.0	-	108.6	104.0	122.7	75.6	111.1

Source: Meteorological station at Kibwezi DRFS, Kadenge Agrometeorological Station (Siaya district)

Appendix 3. Analysis of variance (Anova) of Experiment 3.4.1 for effect of media composition (MC) and quantity (MQ) on growth and sporulation of *C. sesami* and *C. sesamicola*

Source	Degrees of freedom (df)	Mean squares for Colony diameter (mm)		Mean squares for Conidial production ($x10^6$ conidia/ml)	
		C. sesami	*C. sesamicola*	*C. sesami*	*C. sesamicola*
MC	12	698.63 **	6.507 **	78.41 **	411.24 **
Error (a)	26	3.49	0.157	1.70	6.60
MQ	4	54.41 **	0.890 **	13.26 **	16.17 **
MC x MQ	24	1.96 ^{ns}	0.004 ^{ns}	0.85 ^{ns}	1.29 ^{ns}
Error (b)	52	2.42	0.035	0.53	1.35

** Significant at probability level P = 0.01 (F-test)
^{ns} Not significant at P = 0.05 (F-test)

Appendix 4. Anova of Experiment 3.4.2 for effect of media pH (MP) and incubation period (IP) on growth and sporulation of *C. sesami* and *C. sesamicola*

Source	Degrees of freedom (df)	Mean squares for Colony diameter (mm)		Mean squares for Conidial production ($x10^6$ conidia/ml)	
		C. sesami	*C. sesamicola*	*C. sesami*	*C. sesamicola*
MP	3	16072.07 **	13791.96 **	126.78 **	1824.93 **
Error (a)	8	8.33	4.39	0.64	15.93
IP	5	341.83 **	537.95 **	47.93 **	92.46 **
MP x IP	15	23.23 **	30.85 **	6.65 **	16.90 **
Error (b)	40	5.08	4.11	0.50	3.31

** Significant at probability level P = 0.01 (F-test)

Appendix 5. Anova of Experiment 3.4.2 for effect of temperature and Light regime on growth and sporulation of *C. sesami* and *C. sesamicola*

Source	Degrees of freedom (df)	Mean squares for Colony diameter (mm)		Mean squares for Conidial production ($x10^6$ conidia/ml)	
		C. sesami	*C. sesamicola*	*C. sesami*	*C. sesamicola*
Block	2	0.57 ^{ns}	0.15 ^{ns}	2.51 ^{ns}	0.04 ^{ns}
Temperature (T)	4	666.76 **	36.08 **	167.13 **	138.49 **
Error (a)	8	6.07	0.19	4.94	1.26
Light regime (LR)	2	71.51 **	1.67 **	19.49 **	40.39 **
T x LR	8	26.02 **	0.37 **	3.31 **	7.32 **
Error (b)	20	0.34	0.06	0.68	0.40

** Significant at Probability level P = 0.01 (F-test)
^{ns} Not significant at P = 0.05

Appendix 6. Anova of Experiment 3.4.4 for effect of inoculation technique (IT) and subculturing (SC) on growth and sporulation of *C. sesami* and *C. sesamicola*

Source	Degrees of freedom (df)	Mean squares for Colony diameter (mm)		Mean squares for Conidial production ($x10^6$ conidia/ml)	
		C. sesami	*C. sesamicola*	*C. sesami*	*C. sesamicola*
IT	1	6157.17 **	40.21 **	172.74 **	56.48 **
Error (a)	8	1.56	0.33	1.06	6.05
SC	4	30.96 **	11.85 **	10.46 ^{ns}	14.57 *
IT x SC	4	2.62 ^{ns}	3.27 **	47.16	10.22 ^{ns}
Error (b)	32	1.63	0.17	4.60 **	4.12

** Significant at probability level P = 0.01 (F-test)
* Significant at P = 0.05
^{ns} not significant at P = 0.05

Appendix 7. Anova for Area under disease progress curves for percent diseased leaves (AUDPC-DL) and percent defoliation (AUDPC-DF) on 16 sesame accessions infected by *C. sesami* at Kibwezi DRFS and Siaya FTC

Source	Degrees of freedom	Mean squares for experiment at Kibwezi DRFS		Mean squares for experiment at Siaya FTC	
		AUDPC-DL	AUDPC-DF	AUDPC-DL	AUDPC-DF
Block	2	18154.41^{ns}	37366.90^{ns}	37767.73^{ns}	13883.93^{ns}
Accession	15	70061.10**	476502.10**	696179.40**	464836.30**
Error	30	22773.77	19221.62	535669.10	18451.49

** Significant at probability level P = 0.01 (F-test)

^{ns} Not significant at P = 0.05

Appendix 8. Anova for rates of increase in percent diseased leaves (*K*-DL) and percent defoliation (*K*-DF) on 16 sesame accessions infected by *C. sesami* at Kibwezi DRFS and Siaya FTC

Source	Degrees of freedom	Mean squares for experiment at Kibwezi DRFS ($x10^{-4}$)		Mean squares for experiment at Siaya FTC ($x10^{-4}$)	
		K-DL	*K*-DF	*K*-DL	*K*-DF
Block	2	0.112^{ns}	0.044^{ns}	0.738^{ns}	0.024 ns
Accession	15	4.640**	4.120**	3.870**	0.0416**
Error	30	0.511	0.267	1.020	0.072

** Significant at probability level P = 0.01 (F-test)

^{ns} Not significant at P = 0.05

Appendix 9. Anova for Area under disease progress curves for percent diseased leaves (AUDPC-DL) and percent defoliation (AUDPC-DF) on 16 sesame accessions infected by *C. sesamicola* at Kibwezi DRFS and Siaya FTC

Source	Degrees of freedom	Mean squares for experiment at Kibwezi DRFS		Mean squares for experiment at Siaya FTC	
		AUDPC-DL	AUDPC-DF	AUDPC-DL	AUDPC-DF
Block	2	44449.90 ^{ns}	6278.38 ^{ns}	6618.69 ^{ns}	66.53 ^{ns}
Accession	15	484658.60 **	115727.90 **	1088771.00 **	243875.80 **
Error	30	26967.27	6660.45	33462.22	4166.93

** Significant at probability level P = 0.01 (F-test)
^{ns} Not significant at P = 0.05

Appendix 10. Anova for rates of increase in percent diseased leaves (*K*-DL) and percent defoliation (*K*-DF) on 16 sesame accessions infected by *C. sesamicola* at Kibwezi DRFS and Siaya FTC

Source	Degrees of freedom	Mean squares for experiment at Kibwezi DRFS ($x10^{-4}$)		Mean squares for experiment at Siaya FTC ($x10^{-4}$)	
		K- DL	*K*-DF	*K*- DL	*K*-DF
Block	2	1.170 ^{ns}	0.174 **	0.345 ^{ns}	0.204 ^{ns}
Accession	15	2.620 ^{ns}	0.292 **	8.400 **	1.140 **
Error	30	1.540	0.039	1.280	0.078

** Significant at probability level P = 0.01 (F-test)
^{ns} Not significant at P = 0.05

Appendix 11. Ecological requirements of some edible oilseed crops grown in Kenya

Crop	Altitude(m)	Rainfall (mm)	Temp. (° C)	Soil Fertility
Coconut	0-1200	1300-2300	25-29	high
Groundnuts	0-1250	500-1000	25-30	moderate
Sesame	0-1500	300-650	20-27	moderate
Soybean	0-3000	500-750	30-32	moderate
Sunflower	0-2500	500-750	20-25	moderate

Source: Child (1974); Weiss(1983)

Appendix 12. Qualitative comparison of some common vegetable oils

	Fatty acids: percentage by weight									
Source	Myrstic	Palmitic	Stearic	Arachidic	Oleic	Linoleic	Linolenic	Iodine value	Sapon. value	Tocopherol content (mg/g of oil)
Cottonseed	1	21	2	1	25	22	3	108	195	0.92
Groundnut	-	8	4	3	55	25	0	90	189	0.93
Linseed	-	-	9	-	23	20	48	180	192	-
Maize	-	6	2	1	37	54	0	125	191	-
Olive	1	9	1	1	80	8	0	83	192	-
Palm	2	42	4	-	42	10	0	53	198	
Safflower	-	5	1	1	20	70	-	145	191	0.89
Soybean	-	9	2	1	32	53	3	130	192	0.96
Sunflower	-	5	2	1	35	57	0	128	191	-
Sesame	-	8	3	1	47	41	0	111	192	0.66
Rapeseed	-	1	1	-	22	22	3	102	175	-

Source: Weiss (1983)