INHIBITORY EFFECTS OF *Solanum nigrum* L. EXTRACTS ON FOUR PHYTOPATHOGENIC SPECIES OF *Colletotrichum* Corda.

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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This thesis has been submitted for examination with our approval as university supervisors.

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DEDICATION

This thesis is dedicated to my parents Mrs Shelmith Kariba and the late Mr. Kariba M'Naituri, who would have rejoiced at the achievements. My husband Ndege too, takes a share.

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However, I am solely responsible for the manner in which the research was carried out, and how the data were analysed and interpreted.

ABSTRACT.

Title: Inhibitory effects of *Solanum nigrum* L. extracts on four phytopathogenic species of *Colletotrichum* Corda.

In this study *Solanum nigrum* extracts were evaluated for their potential as a source of a biologically based fungicide. The extracts were tested *in vitro* against four phytopathogenic species of *Colletotrichum* Cda. The fungi included *C. coffeanum* Noack, *C. lindemuthianum* (Sacc. and Magn.) Schribner, *C. musae* Berk. and M.A Curtis and *C. gloeosporioides* (Penzig.) Sacc. These were isolated from coffee berries, bean pods, ripe bananas and mango fruits respectively. The pathogens were isolated from their hosts and cultured in potato dextrose agar (PDA).

Solanum nigrum plants were grown in October, 1990 from seeds collected from gardens around Nairobi. The plants were harvested at four week intervals and taken to the laboratory where they were separated into different parts viz: roots, stems, leaves and fruits. Water extracts were made from the different plant organs and tested on the four species of *Colletotrichum*. Some of the extracts were kept in the freezer for later use.

The effect of different concentrations of extracts was determined by diluting the extracts using sterile distilled water. Each aqeous extract was mixed with sterilized PDA cooled to 40 °C, and this was then dispensed into petri dishes. Each extract and every concentration level had four replicates. Sterile distilled water was used in place of the extract for the control. Colony diameters were measured daily for a period of 9 days. The colony diameters on the treated plates compared to that on the control was taken as a relative measure of fungitoxicity.

Mycelial growth and sporulation of the pathogens was less in media treated with *S. nigrum* extract than on the control plates. Extracts from different plant parts had different inhibitory effects. Leaf extracts were found to have the highest inhibitory effects as compared to extracts from other plant parts.

Higher concentrations of the extract were found to be more effective than the lower ones. This was true for stem, root and fruit extracts. Inhibition by leaf extracts did not vary with dilution upto the level used in this study.

Extracts from plants at different ages had varying inhibitory effects whereby extracts from three months old plants were found to be most inhibitive. However, inhibition by leaf extracts did not vary with plant age. Extracts from fruits at different stages of development had varying inhibitory properties. The green fruit extracts had higher inhibition than those from the red ripe fruits.

The differences in response to *Solanum nigrum* extracts by the four species of *Colletotrichum* were not statistically significant ($p>0.05$).

From this study, there is sufficient grounds for the conclusion that *Solanum nigrum* may be a potential source of a fungicide against crop diseases caused by the four species of *Colletotrichum.*

CHAPTER 1

1.0 INTRODUCTION

Agriculture is the mainstay of Kenya's economy and over 85% of Kenyans derive their livelihood from this activity (Republic of Kenya, 1989). It follows therefore that increased crop production has important implications for Kenya's overall development, and better living standards especially for the rural community.

There are various problems that plague efficient crop production in Kenya. Such problems include crude farming technologies, unreliable weather, and, crop pests and diseases. The latter is of great economic importance and accounts for a high percentage of crop losses in Kenya (Acland, 1971). Protection of crops from pests and diseases should be given serious consideration in order to obtain stable production of agricultural products (Reynolds, 1985).

It is, therefore, evident that without the contributions that pesticides make to modern agriculture, the cost of food would skyrocket with negative impacts on those societies that are not economically advantaged. Exposed to the uncontrolled ravages of fungi, bacteria and various insects, both food and cash crops would be destroyed in the farms, and post harvest losses would also occur before the products are consumed or marketed (Todhunter, 1985).

This study focuses on the control of a serious disease affecting some economically important crops in Kenya, caused by species of *Colletotrichum* Corda. The genus *Colletotrichum* is characterised by septate branched mycelium with saucer - shaped acervuli bearing simple elongate conidiophores; conidia hyaline, 1 - celled, ovoid or oblong (Sutton, 1980; Alexopoulus and Mims, 1979; Barnett and Hunter, 1972). The genus belongs to the form - class Deuteromycetes of the form - order Melanconiales. Some species of the

genus *Colletotrichum* cause diseases known as anthracnose on *Coffea arabica* L. (coffee), *Phaseolus vulgaris* L. (beans), *Musa sapientum* L. (bananas) and *Mangifera indica* L. (mangoes) among others.

The species of *Colletotrichum* responsible for causing anthracnose on these crops are; *Colletotrichum coffeanum* Noack, *C. lindemuthianum* (Sacc. and Magn.) Scribner, *C. musae* Berk. and M.A Curtis. and *C. gloeosporioides* (Penzig) Sacc. respectively. These fungi are responsible for great losses in the production of these crops (Acland, 1971).

1.1 Coffee anthracnose

Coffea arabica (coffee) is a leading foreign exchange earner in Kenya. The major constraint to economic production of arabica coffee in Kenya has been coffee berry disease (CBD) caused by *C. coffeanum*. The fungus infects flowers, green and ripening berries and rarely leaves. Two groups of symptoms have been distinguished (Kiragu, 1983). Anthracnose of leaves and fruits characterised by lesions and dieback which is desiccation of the extremities of branches. The various symptoms have been described in details by various workers (Mulinge, 1970; Acland, 1971). Great losses occur where CBD is reported. The disease usually causes pulping difficulties and bean staining, thus, resulting in loss of quality (Hocking, 1966). Losses could be as high as 80 per cent (Griffiths *et al.* 1971)

1.2 Bean anthracnose

Phaseolus vulgaris L. (beans) is an important food crop and a major source of proteins for the farming community in Kenya (Acland, 1971). Currently small acreages are planted with Mexican 142 variety for the export market. Yields are however low due to land scarcity,

competition from other crops, and, diseases and pests (Khamala, 1978). Anthracnose caused by *C. lindemuthianum* is an important foliar disease occurring in all bean producing areas of the world (Hubbeling, 1957; Leaky, 1970; Roberts and Boothroyd, 1972; Acland, 1971; Mutitu, 1979). Crop losses due to *C. lindemuthianum* have been reported in all bean growing areas.

Losses of nearly 100 per cent occur in areas of high humidity when diseased seeds are planted (Chaves, 1980). In addition to infection in beans, (*Phaseolus vulgaris* L.) the pathogen has also been reported to cause losses in lima beans (*Phaseolus lunatus*) L., mang beans (*P. aureus* (L) Wilczek., broad beans (*Vicia faba* L.) and Kudzu beans (*Delichos biflorus* Linn.) (Walker, 1969; Roberts and Boothroyd, 1972).

1.3 Banana anthracnose

The banana (*Musa* spp.) is widespread in Kenya. The fruit is one of the most popular and least expensive fruit available in the world markets. It is a nutritious and wholesome fruit that forms an important component in the diet of tropical third world countries. In developed countries, bananas are marketed as fresh fruits and as chips (Slabaugh and Grove, 1982).

Bananas are grown in Kenya mostly as a subsistent crop although there is much internal trading between banana growing areas and main towns. Banana anthracnose caused by *C. musae* is an important disease of ripe and ripening fruits. The fungus is widely distributed and important in all producing countries (Snowdon, 1990). It is important as a wound pathogen but also attacks unwounded fruits resulting in decay that leads to great post - harvest losses.

1.4 Mango anthracnose

The mango (*Mangifera indica* L.) is an important tropical fruit. In Kenya, mangoes are a minor export crop but a very important fruit (Acland, 1971). Mangoes are very susceptible to anthracnose caused by *C. gloeosporioides* (Snowdon, 1990; Jacobs *et al*, 1973). In addition to mango infection, the pathogen has been observed to cause losses in pawpaw (*Carica papaya* L.), Citrus fruits (*Citrus sinensis* Pers.), cocoa (*Theobroma cacao* L.), Avocado (*Persea americana* Mill.) and in guavas (*Psidium guajava* L.) (Stanghelini and Aragaki, 1966; Snowdon, 1990; Brown, 1975; Denham and waller, 1981; Mohanan *et al*, 1989; Chatrath *et al*, 1968; Cappellin *et al* 1988; Fagan, 1979).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Control methods

Much research work has been done on the control methods of anthracnose diseases caused by species of *Colletotrichum* and various fungicides have been recommended (Vine *et al*, 1973a; Chaves, 1980; Ram, 1983; Tandon and Singh, 1968b).

2.1.1 Coffee berry disease (CBD)

In Kenya, Coffee berry disease caused by *Colletotrichum coffeanum* is controlled by the use of resistant varieties and fungicides (Gibbs, 1968; Vine *et al*, 1973a; Mulinge and Griffiths, 1974; Nutman and Roberts, 1970).

Since the introduction of coffee in E. Africa by missionaries in 1880 (Jonnes, 1956) considerable work on selection and hybridization has been done. Among *Coffea arabica* cultivars, Geisha and blue mountain have been known to be rather resistant to coffee berry disease in East Africa (Rayner, 1952). Cultivar K_7 showed appreciable resistance (Gibbs, 1968). Techniques used for screening included the detached berry test as well as field observations on natural disease incidence (Bock, 1956).

By 1952 it was known that some degree of control could be achieved by fungicide sprays (Rayner, 1952). Since then several workers have carried out research on the control of (CBD) using fungicides by laboratory screening followed by field trials (Bock, 1956, 1963; Nutman and Roberts, 1970; Furtado, 1969; Mulinge *et al*, 1974 and Vine *et al*, 1973a).

By 1973, many protectant fungicides that had been identified gave 80 - 90 per cent control of CBD even during severe epidemics (Vine *et al*, 1973b; Cook, 1975, Cook and Pereira, 1977; Okioga, 1975). There is therefore need for research on new effective CBD fungicides.

2.1.2 Bean anthracnose

For the control of bean anthracnose, attention is given to various practices. These include the use of pathogen free seeds (Walker, 1969; Tu, 1981), early removal and destruction of infected seedlings, field selection of anthracnose free pods and crop rotation to avoid residual contamination (Heald, 1943; Roberts and Boothroyd, 1972; Cook, 1978).

Seed treatment and foliar sprays with various suggested fungicides such as thiram and ferbam have been used to control the disease (Sindhan and Bose, 1981; Cook, 1978; Chaves, 1980; Tu, 1981). Mancozeb (Dithane M45) and metivam (polyram) combinations are commonly used in Kenya for the control of anthracnose (Kinyua, 1976/77b). These measures cannot eradicate the disease but can reduce yield loss. The fungus for example develops resistance to some fungicides used for seed treatment and new races of the pathogen appear from time to time (Tu, 1981; Tu and Aylesworth, 1980; Leaky *et al*, 1978).

The success of bean anthracnose control, therefore, rests on the use of resistant varieties (Yerkes, 1958; Leaky, 1970). However, success of production of resistant strains by crossing is complicated by the number of physiologic strains (Heald, 1943). Therefore, there is need for new effective fungicides.

2.1.3 Banana anthracnose

The control of banana anthracnose include picking of the fruit at the right stage of maturity, careful handling during local transport to minimise bruising, organisation to limit the period of exposure of infected wounds to tropical temperatures, and cleanliness in collecting stations and ripening rooms (Snowdon, 1990). The disease can also be reduced by use of fungicides (Muredith, 1963; Ram, 1983; Cook, 1975).

Slabaugh and Grove, (1982), found that washing bananas in chlorinated water could reduce banana anthracnose. Benomyl application in banana plantations also reduced the disease. Some banana cultivars have also been reported to be resistant to *C. musae* (Shillinford, 1977).

2.1.4 Mango anthracnose

This disease can be controlled both by pre- and post-harvest measures. The disease can be fairly well controlled during flowering and early fruit development by using fungicide sprays (Denham and Waller, 1981; Tandon and Singh, 1968b; Snowdon, 1990). Careful harvesting of fruits and packing during storage is recommended to avoid injury (Tandon and Singh, 1968b).

The most usual post-harvest treatment involves immersion of the fruit in hot water containing a fungicide. However time and temperature are critical if anthracnose is to be controlled without heat injury to the fruit (Smoot and Segall, 1963; Tandon and singh, 1968a; Pathak and Shekhawat, 1976; Siboe, 1982; Spalding and Reeder, 1986).

2.2 Use of fungicides for plant disease control.

Fungicides are employed on one-half of the world crops though with some disadvantages (Lukens, 1971). Fungicides like all pesticides are biologically active and if used in an uncontrolled fashion, can be hazardous to humans and the environment (Mandava, 1985; McEwen and Stephenson, 1979). Some are acutely toxic, non biodegradable and others have posed threats such as inducing resistance or tolerance in pathogens (Mandava, 1985). *Colletotrichum* spp. have for example shown resistance to fungicides, rendering some obsolete. *C. coffeanum*, *C. gloeosporioides* and *C. musae* have shown tolerance to benomyl and it is, therefore, no longer used for their control (Javed, 1980; Mwang'ombe *et al*; 1988; Griffee, 1973; Spalding, 1982).

Natural products of plant origin are a vast source of bioactive substances that have been exploited only to a limited extent (Menn, 1983). There are some plants that are attacked by few parasites. Disease resistance in these plants is known to be due to the presence of certain chemical substances in the host tissue that are toxic to some specific pathogens (Lwande, 1984). Some members of the families *Solanaceae*, *Amaryllidaceae* and genus *Digitalis* are rarely attacked by parasites. A majority of these plants are known to contain alkaloids (Meeuse, 1973). These alkaloids are probably responsible for inhibiting the parasites.

The genus *Solanum* in the family *Solanaceae* is generally characterised by the bitter glycoalkaloid called Solanine, first isolated from *Solanum nigrum* (Watt and Breyer-Brandwijk, 1962; Schreiber, 1968).

Solanum nigrum offers a possibility for a supplement fungicide. This is based on the

earlier medicinal uses the plant has been put to, for example, the treatment of ringworm (a fungal infection) in Kenya, India and South Africa (Jain, 1979; Watt and Breyer-Brandwijk, 1962).

S. nigrum is mainly regarded as a weed. It is a branched annual herb with alternate simple rather dark leaves, ovate and pointed at the tips. It has small clusters of star shaped white flowers with yellow centres. The fruit is a many seeded globular berry, usually turning to red, orange or black when ripe (Ivens, 1967; Muenscher, 1951; Salisbury, 1961; Tampion, 1977; Tindall, 1983; Holm *et al*, 1979).

The plant is widely distributed and is found in the tropics and most temperate countries. It is widespread throughout East Africa growing from sea level to at least 7000 feet above sea level (Ivens, 1967). The plant is very common in arable land and sometimes troublesome in gardens with rich loamy soils (Salisbury, 1961). It is also common in waste grounds (Ivens, 1967).

Solanum nigrum has been put to various uses in different parts of the world. The leaves are eaten as vegetables in E. Africa, W. Africa, Ethiopia and Zimbabwe (Bailey, 1973; Schery, 1972; Verdcourt and Trump, 1969; and Ivens, 1967). The ripe berries are eaten as fruits and used in jam making in U.S, Southern Canada and Zimbabwe (Muenscher, 1951; Ivens, 1967).

For Medicinal purposes the plant is used in many countries, for example, in the treatment of ringworm and a remedy for abdominal pains in Kenya, India and S. Africa (Watt and Breyer-Brandwijk, 1962; Kokwaro, 1976). *S. nigrum* is used in the treatment of Asthma, urinary diseases, and excessive thirst. It has also been cited as an important ingredient in several indian medicines (Jain, 1979).

2.3 Pesticides of plant origin

Future pest control methods may include potent, more selective and biodegradable pesticides discovered as natural products from higher plants. A number of botanical pesticides like pyrethrins, rotenoids, nicotine, the natural isobutylamides, quassia and ryania have been approved for commercial uses (Gunther and Jeppson, 1960; Mandava, 1985). Success to date has come from scrutiny of medicinal plants which were remedies of established efficacy but unexplained mode of action (Gunther and Jeppson, 1960). The technique employed is, first, the identification of plants for extended phytochemical studies by evaluating their crude extracts for pesticidal properties (Farnsworth, 1973).

Various workers have reported investigations of antimicrobial activities of plant extracts. Awuah (1989) evaluated extracts from five West African plants *in vitro* for their fungitoxicity against *Ustilago maydis* (DC) Corda., *Ustilaginoidea virens* (CKe) Takahashi., *Curvularia lunata* (Wakker) Boedijn., and *Rhizopus* spp. Steam distillates from leaves of *Cymbopogan citratus* (Link.), completely inhibited growth of all four fungi. Hot water extracts from leaves of *Occimum gratissimum* (Forsk.) and *Chromoleana odorata* (DC), and dry fruits of *Xylopia aethopica* (Rich.), reduced mycelial radial growth of the test fungi by 10 - 60 per cent.

Bynum (1965) investigated the effects of incense cedar heartwood extract on growth of *Polyporus amarus* and experienced reduced mycelial quality with increased extract concentration. The reduction of growth of *Polyporus amarus* by incense cedar heartwood extract suggested its fungitoxic nature.

Chiappeta *et al* 1988, screened *Solanum viarum* (Dun.) extracts in the laboratory and found the extracts to have both antibacterial and antifungal activities. They also found

extracts from different parts of the plant to have varying inhibitory effects.

Different parts of *Aframamum melegueta* (Rosc.) K. shum., a perennial herb cultivated in tropical Africa, have been used for the preparation of folk remedies. Oloke *et al* (1988) carried out investigations with *A. melegueta* extract on some species of bacteria and fungi and found the extract to have both antibacterial and antifungal effects.

The juice of *Allium sativum* L. (garlic), aqueous as well as certain organic solvent extracts posses strong bacteriocidal and fungicidal properties. Ark and Thompson (1959) found that downy mildews of cucumber, bean rust, bean anthracnose, early blight of tomato and bacterial blight of beans were effectively controlled by aqueous extracts of garlic powder preparations.

Wood extracts have effective fungitoxic activities and have been shown to inhibit several fungi including human pathogens. *Catalpa speciosa* (warder.) for example has yielded a water soluble material and an alcoholic extract which has been shown to inhibit the growth of some wood decaying fungi (McGray and Macdonough, 1954).

In vitro tests with cultures of *Polyporus versicolor* and *Poria monticola* showed that *Quercus alba* Linn. extract is fungitoxic (Hart and Hillis, 1972).

In an effort to develop a simple and effective media for three fungal pathogens of rice, *Pyricularia oryzae*, Thrum, Kulkarni and Patel., *Corticium sasakii* and *Drechslera oryzae*, leaf extracts of 23 plants were tested. In this study it was found that some leaf extracts inhibited mycelial growth of the pathogens (Tewari and Premalatha, 1984).

Shekhawat and Prasada (1971) tested cold water extracts and acetone extracts of different parts of 41 plant species from 28 families against three plant pathogenic fungi and found several of them to have antifungal properties. In most cases leaf extracts were used but those from pods, roots and bark were also used. All the plant parts had some antifungal

effects.

Root extracts of *Cymopsis tetragonoloba* (guar) have been shown to reduce the growth rate of root rot pathogens of wheat, viz. *Colletotrichum graminicola* (Ces.) Wilson., *Fusarium moniliforme* Sheldon and *Cladosporium* spp (Johnson, 1980).

In the study of the effect of some plant extracts on conidial germination of *Curvularia pallescens* Boedijn, Kumar and Sachan (1979) found that extracts from different plants had varying inhibitory effects on the fungus. It was also found that extracts from different parts of the plant had varying inhibitory effects. No conidial germination occurred in the root extract of *Dioscorea sativa* (Beatson), while in the case of leaf extracts of *Tagetes erecta* maximum germination (100 per cent) was observed. *Narcicus* spp leaf extracts gave 76 per cent conidial germination. This therefore indicated that extracts from different plants and from different parts of the plants had varying effects on *C. pallescense* Boedijn.

However, it was found that although there was conidial germination in some extracts, germ length was short in all extracts in comparison to that of the control.

Leaf extracts from ten medicinal plants were made and tested on *Curvularia lunata* and found to vary in their effects. Some extracts completely checked the radial mycelial growth of the test fungus and some extracts had no effects. Mycelial dry weight of the test pathogen was also reduced in varying proportions after treatment with all the leaf extracts. These findings were attributed to the presence of certain antifungal compounds in the leaf extracts of some plant species (Bhowmick and Vardhan, 1981).

During the screening of various plant extracts for antifungal properties, Kumar and Nene (1968) found extracts of *Cleome isacandra* to posses strong antifungal properties. Extracts from different parts of the plant (leaves, roots, stems and flowers) completely inhibited the growth of *Helminthosporium maydis* Nishikado and Niyake, *H. turcicum Pass.*,

Alternaria solani (Ell. and mart.) Vones and Grout., *Glomerella cingulata* (Stone) Spaulding and V. Schrenk. and *Sclerotum rolfsil* Sacc. The growth of *Aspergillus niger* Van Tiegh, and *Rhizopus nigricans* Ehrenb was however not inhibited.

Plants belonging to the family Lamiaceae are reputed for their medicinal uses. In 1988, Diaz and his colleagues evaluated 43 species of plants representing 14 genera of the family lamiaceae. It was found that 36 per cent exhibited inhibitory effects against at least one test micro-organism.

In 1976 extracts of *Solanum incanum* were proven to have inhibitory effects against bacteria and fungal pathogens including human pathogens and phytopathogenic fungi (Mbaya and Muhammed, 1976). Hexane extracts derived from roots and above ground parts of Mississippi indigenous plant *Hypericum drummondi* Torr. and Gray, had significant activity against gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* (Hiranthi *et al*, 1989).

Apart from the evaluation of inhibitory effects of plant extracts on bacteria and fungal pathogens there has also been similar studies on inhibitions of extracts from higher plants on viruses. Smookler (1971), evaluated 29 species of *Chenopodiales* for their antiviral action. It was found that many of these extracts inhibited the infection of *Phaseoulus vulgaris* by *Tobacco necrosis* virus (TNV).

In 1984, Pandey and Bhargara evaluated the inhibitory effects of *Ampelopteris prolifera* extracts on *tobacco mosaic* and *cucumber mosaic* viruses and found the two viruses to be inhibited.

In 1947, Kuntz and Walker found spinach (*Spinacia oleracea* L) extracts to posses inhibitory effects towards viruses. The viruses were more inhibited by the same extract at higher concentrations than at low concentrations. Hence, there was direct effect of dilution the spinach extract upon the inhibitory property.

Research on new effective fungicides and investigations on reported or suspected resistance of *Colletotrichum* to some of the recommended chemicals is inevitable. This project is aimed at exploring the possibility for additional fungicides.

Development of plant extracts that are inhibitory to fungi affecting economic crops in Kenya would help towards increasing crop yields and thus boosting the country's economy. This is especially so if an extract can be obtained from a fast growing plant and thus can be produced quickly and at minimum cost. After the biological effect of the crude extract is evaluated, further work can be carried out on the active ingredients of the extracts. In the present study, antifungal activity of extracts from *Solanum nigrum* were investigated. Water extracts from different parts (leaves, stems, roots and fruits), and ages of the plant were tested against four phytopathogenic species of *Colletotrichum.*

2.4 Objectives

The major objectives of this study were:

1) To investigate the inhibitory effects of *Solanum nigrum* extracts against four phytopathogenic species of *Colletotrichum.*

2) To investigate the part of the plant (*S. nigrum*) with the highest inhibitory effects against the four phytopathogenic *Colletotrichum* species.

3) To investigate the age of *S. nigrum* at which optimum inhibitory effects against the four fungal species is obtained.

4) To investigate the effects of dilution of *S. nigrum* extracts on the inhibitory properties.

5) To determine whether there is a difference in inhibition among the four phytopathogenic species of *Colletotrichum.*

CHAPTER THREE

3.0 MATERIALS AND METHODS

Solanum nigrum was grown from seeds collected from gardens around Nairobi in October 1990. The seeds were germinated in a nursery and later planted in lines on a 10 x 10 m plots at Chiromo campus. Cow manure was used to enrich the soil where the seedlings were planted. These were watered daily for two weeks. Watering was then continued at two day intervals until the plants were one month old. After the age of one month, watering was done once a week.

3.1 Fungal pathogens

The fungi used in the study were *Colletotrichum coffeanum, C. lindemuthianum, C. musae* and *C. gloeosporioides*. These were isolated from coffee berries, bean pods, ripe banana peels and mango fruits respectively. The pathogens were cultured in potato dextrose agar (PDA). In cases where there was a lot of saprophytic growth, the pathogens were cultured in water Agar (WA) then transfered into PDA. The four species were identified using the key to species of *Colletotrichum* by Sutton (1980).

3.1.1 Methods of media preparation.

The media were prepared according to the procedure outlined by Smith and Onions (1983) appendix (1).

3.1.2 Isolation of *Colletotrichum coffeanum*

Ripe coffee berries with dark sunken patches and minute black dots were collected from a coffee farm near Riruta Satellite shopping centre in Nairobi and brought to the mycology laboratory. The coffee berries were washed with tap water and placed in petri dishes lined at the bottom with moist filter paper. This was done according to Barbara (1991). After 24 hrs several pinkish conidial ooze were visible on the lesions. These were gently removed with a sharp sterile needle and streaked on Potato dextrose agar plates and kept at room temperature. Mycelial growth was visible on the plate on the third day of incubation. This was subcultured until a clean culture was obtained. The culture was then kept for 5 - 7 days for sporulation to occur.

3.1.3 Isolation of *Colletotrichum lindemuthianum*

Bean pods with dark brown sunken circular lesions were collected from Kabete farm and brought to the laboratory for analysis. The infected bean pods were washed with tap water, surface sterilized with 10 per cent sodium hypochlorite, rinsed with sterile distilled water, and dried between sterile filter papers. The lesions bearing parts were cut with a sterile scapel blade into small pieces then transferred into PDA plates. The plates were sealed with parafilm wax and incubated at room temperature. Pure colonies of *C. lindemuthianum* were obtained after several subcultures had been done.

3.1.4 Isolation of *Colletotrichum musae*

Ripe banana fruits with dark circular sunken spots were bought from Nairobi City market and brought to the laboratory. The fruits were washed under running tap water for one hour and lesions bearing portions were cut into small pieces and immersed into 10 per cent Sodium hypochlorite for 2 minutes. The pieces were then removed and immersed into sterile distilled water to wash off the excess bleach and then transferred onto PDA media. The plates were sealed with parafilm wax paper and incubated at room temperature. The resulting mycelia were transferred into clean PDA plates. This was repeated until a clean culture was obtained.

3.1.5 Isolation of *Colletotrichum gloeosporioides*

Infected mangoes with purplish lesions were bought from Nairobi city market and brought to the laboratory for studies. The mangoes were washed under running tap water for one hour. Excess water was removed by blotting it with filter papers. Lesions on the mangoes were cut into small pieces, immersed into 10 per cent sodium hypochlorite for 2 minutes and then rinsed with sterile distilled water. The sterile pieces were then transferred into PDA plates, sealed with parafilm wax paper and incubated on laboratory benches. After three days mycelial growth was visible on the plates. This was subcultured into clean PDA plates until a clean culture was obtained then incubated and allowed to sporulate.

3.2 Preparation of plant material

Solanum nigrum plants grown at chiromo botanical garden were harvested at four week intervals. The plants were uprooted at random from the plots then taken to the laboratory and separated into different parts as follows; roots, stems, leaves and fruits. This was done according to Bynum (1965), Kumar and Sachan (1979), Awuah (1989), Oloke (1988), Shekawat and Prasada (1971) and Chiappeta *et al* (1988).

The two and three month old plants, leaves and stems were further subdivided into L^1 and L^2 where L^1 was the very young leaves and L^2 the older leaves. The stems were divided into S^1 and S^2 , the younger and older stem respectively. The roots were not subdivided.

For the four month old plants the leaves were divided into L^1, L^2 and L^3 based on age in increasing order. The stem was divided into S^1 and S^2 just like for the two months and three months old plants. At this age fruits had formed. The fruits were divided according to their stages of development. The divisions were F^1, F^2 and F^3 where F^1, being the youngest, F^2 refers to the mature unripe green fruits and F^3 the mature ripened fruits.

3.2.1 Extract preparation

Fresh plant material was weighed separately into required quantities. The plant material was first washed with running tap water for one hour, it was then surface sterilised with 10 per cent sodium hypochlorite for 20 minutes. The material was then rinsed with sterile distilled water and crushed with a mortar and pestle containing a known volume of sterile distilled water.

Cold water extraction as by Shekhawat and Prasada, (1971) was done. The extracts were then filtered through double layers of cheese cloth into a sterile conical flask as per Shekhawat and Prasada (1971), Pandey and Bhargara (1984) and Awuah (1989).

The filtrate was further filtered through a layer of sterile cotton wool in a filter funnel. This completely removed the solid debris thus making the extracts ready for bioassay.

3.2.2 Concentration levels

The effect of different concentration of *Solanum nigrum* extracts on the radial mycelial growth of the four species of *Colletotrichum* was determined on potato dextrose agar medium amended with extract at concentration levels of 0.025, 0.033, 0.05, 0.1 and 0.2 grams per millilitre of water.

To 100 grams of crushed material, 500 mls, 1000 mls, 2000 mls, 3000 mls and 4000 mls of sterile water was added to make 0.2 g/ml, 0.1 g/ml, 0.05 g/ml, 0.033 g/ml and 0.025 g/ml respectively. This was done according to Johnson (1980).

3.3 Bioassay

Twenty-five millilitres of each aqueous extract (0.2 g/ml, 0.1 g/ml, 0.05 g/ml, 0.033 g/ml and 0.025 g/ml) was mixed with fifty millilitres of autoclaved potato dextrose agar cooled to 40° C. This was then dispensed into petri dishes and allowed to solidify under the microflow chamber in accordance with Shukla *et al* (1972), Johnson (1980), Torgeson (1967) and Awuah (1989).

For each extract and at every concentration level, four replicates were made. As for

the control plates twenty-five millilitres of sterile distilled water was added to fifty millilitres of potato dextrose agar.

The volume of PDA used in the petri dish bioassay was constant throughout the experiment. Thus, the effect of media on the dilution of extract did not affect the experimental results.

3.3.1 Inoculation

Using a sterile cork borer, 8 mm diameter mycelial plugs were cut from the margins of actively growing cultures of the test fungi. With a sterile scapel one plug was transferred asceptically and placed upside down in the centre of each of the PDA plates containing the plant extracts and also to the control plates. Innoculum from cultures grown in water agar was used so as to avoid transfer of nutrients via the innoculum plugs.

The plugs were kept in an inverted position so that the fungi were in direct contact with the treated media as per Shukla *et al* (1972), Reilly and Lamoureux (1981) and Nene (1971).

Plates were sealed with parafilm wax paper and incubated on laboratory benches at room temperature (23 - 27)° C. Colony diameters were recorded daily. The measuring was done on the same axis throughout the experiment unless there was a contaminant (Thitai, 1982). Colony diameters were recorded till fungal growth in one of the treatments filled the petri dish plate. Sporulation was recorded at the end of the incubation period (Tewari and Premalatha, 1984). This was recorded under three different gradations viz (-) nil, (+) poor, and (++) good judged by visual observations. This was done according to Bhowmick and Vardhan (1981); Siboe (1982). Radial growth was determined by measuring the four replicate colonies and subtracting the diameter of the inoculum plug (Smith, 1978). The colony diameter of the treated plates compared with that of the control was taken as a relative measure of fungitoxicity.

The procedure was repeated with extracts from all parts of the plant along with the

four fungi. This treatment was applied to *S. nigrum* for the plants at 4 weeks interval until maturity.

Growth inhibition was calculated as a percentage of the control using the formulae;

$$\%\text{inhibition} = \frac{C - T}{C} \times 100$$

where C is mycelial growth in the control plates

T is mycelial growth in the treated plates.

For statistical analysis the value $\frac{C - T}{C} \times 10$ was used.

Data was analysed by ANOVA after which a multiple comparison test (Tukey) as outlined by Zar (1984) was performed.

CHAPTER FOUR

4.0 RESULTS

4.1 *Colletotrichum* species

Four species of *Colletotrichum* viz *C. coffeanum, C. lindemuthianum, C. musae* and *C. gloeosporioides* were isolated from ripe coffee berries, bean pods, ripe banana fruits and mango fruits respectively.

In potato dextrose Agar (PDA), *Colletotrichum coffeanum* formed pale brown mycelial colonies with lighter centres. The colonies were observed to turn white after subculturing for four times. Hyaline, straight, cylindrical, aseptate conidia were formed directly from the mycelium, measuring 12.8 - 18.3 x 3.58 - 4.58 μm, with a mean length of 15.6 $\pm$ 1.7 μm and a mean width of 4.13 $\pm$ 0.56μm. These measurements are within the ranges that have been reported by other workers (Sutton, 1980; 12.5 - 19 x 4 μm). The mean radial growth rate for a culture incubated for ten days was found to be 0.98 cm/day. *Colletotrichum lindemuthianum* had light grey mycelia that turned black at maturity in PDA. Hyaline cylindrical conidia were formed at conidiophore tips, measuring 11.89 - 18.3 x 3.66 - 5.4 μm, with a mean length of 15.3$\pm$ 2.1 μm and a mean width of 4.2 $\pm$ 0.6 μm. These measurements are within the ranges that have been reported by other workers 11 - 20 x 2.5 - 5.5μm (Mordne, 1971). *C. lindemuthianum* had the slowest growth rate as compared to the other species. It was therefore distinguished from the others by it's slow growth rate (0.63 cm/day), and dark pigmentation in culture. *Colletotrichum musae* formed white to light grey mycelial colonies in PDA. Aseptate hyaline conidia were produced directly on the mycelium, measuring 11.89 - 16.7 x 4.58 -

5.4 μm, with mean length of 14.39 $\pm$ 1.4 μm and mean width of 4.2 $\pm$ 0.5μm. These measurements are within the ranges that have been reported by other workers (Sutton 1980; 12 - 17 x 4.5 - 5.5μm, Simmonds, 1965; 12.2 - 14.6 x 4.9 - 5.8μm, Sutton and Waterson, 1970; 11 - 17 x 3 - 6(4.5μm)). The culture had a fairly high radial growth rate of 0.9 cm/day.

Colletotrichum gloeosporioides formed greyish white mycelium turning to dark grey. Hyaline aseptate cyrindrical Conidia were formed, measuring 10.98 - 21.96 x 3.58 - 4.76 μm, with a mean length of 14.3 $\pm$ 3.1μm and a mean width of 4.1 $\pm$ 0.64μm. These measurements are within the ranges that have been reported by other workers (Sutton, 1980; 9 - 24 x 3 - 4.5μm, Mordne, 1971; 9 - 24 x 3 - 6μm, Simmonds, 1965; 11.9 - 17 x 3.6 - 5.8μm, Roy, 1982; 13.3 - 22 x 3.6 - 4.8μm). The radial mycelial growth rate was 0.8 cm/day.

Colletotrichum musae had slightly longer and broader conidia as well as more rapid mycelial growth in culture than *C. gloeosporioides*. In this regard sutton and Waterson, 1970; observed *C. musae* to grow more rapidly in culture at 24° C than *C. gloeosporioides*.

4.1 *Solanum nigrum*

Solanum nigrum seeds germinated five days after sowing. Plate 1 (a - d) shows the plants at different ages and Plate 1(e) shows a pure stand of the plant growing at the Chiromo botanical garden.

Plate 1(a). one month old *Solanum nigrum*.

Plate 1(b).Two months old *Solanum nigrum.*

Plate 1(c) Three months old *Solanum nigrum* with star-shaped white flowers with yellow centres.

Plate 1(d) Four months old *Solanum nigrum* with flowers, green and ripe fruits.

Plate 1(e) A pure stand of two months old *Solanum nigrum* at the Chiromo botanical garden.

4.2 Effects of *Solanum nigrum* extracts on mycelial growth and sporulation of the four species of *Colletotrichum*

The radial mycelial growth of all the test fungi was found to be less on all the media treated with plant extracts than on the controls (Table 1-4). However, this was also dependent on extract concentration. At low concentration levels, the fungal colony diameters were larger than those on plates treated with higher concetrations of the extracts. This was however not true for the leaf extracts up to the concentration level used in the study (Table 1 - 4 and fig. 2-5). Radial mycelial growth and sporulation on the treated plates compared to that on the control was taken as a measure of effectiveness of the extracts. Fungi in the treated plates did not sporulate except in a few cases where sporulation was minimal (Table 1 - 4). Apart from having smaller colony diameters and minimal or no sporulation, growth rates were also suppressed by the extracts (Table 5). Thus *Solanum nigrum* extracts inhibits or rather supresses growth and sporulation of the four species of *Colletotrichum.*

Table 1(a). Effects of *Solanum nigrum* extracts from one month old plants on mycelial growth and sporulation of *Colletotrichum coffeanum.*

Extract	conc. (g/ml)	colony diameter(cm)		%inhibition	sporulation (in grades)
Leaf		Mean	S.E		
	0.0	7.7	0.07	0	++
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem					
	0.0	7.7	0.07	0.0	++
	0.025	3.6	0.04	53.5	-
	0.033	3.1	0.10	59.7	-
	0.05	2.6	0.02	66.2	-
	0.1	1.6	0.03	78.8	-
	0.2	0.6	0.04	92.2	-
Root					
	0.0	7.7	0.07	0.0	++
	0.025	4.5	0.08	41.6	+
	0.033	4.0	0.12	48.1	-
	0.05	3.4	0.03	56.1	-
	0.1	2.5	0.01	67.5	-
	0.2	1.5	0.04	80.5	-

Table 1(b).Effects of *Solanum nigrum* extracts from one month old plants on mycelial growth and sporulation of *Colletotrichum gloeosporioides.*

Extracts	conc. (g/ml)	colony diameter(cm)		%inhibition	sporulation (in grades)
Leaf		Mean	S.E		
	0.0	6.2	0.06	0	++
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem					
	0.0	6.2	0.06	0.0	++
	0.025	3.4	0.03	44.7	-
	0.033	2.9	0.05	53.2	-
	0.05	2.4	0.09	61.3	-
	0.1	2.6	0.04	74.5	-
	0.2	0.5	0.07	91.9	-
Root					
	0.0	6.2	0.06	0.0	++
	0.025	4.4	0.07	29.0	+
	0.033	3.9	0.02	37.1	+
	0.05	3.1	0.09	50.0	-
	0.1	2.3	0.03	62.4	-
	0.2	1.3	0.09	79.0	-

S.E - Standard Error

Table 1(c). Effects of *Solanum nigrum* extracts from one month old plants on mycelial growth and sporulation of *Colletotrichum Musae.*

Extracts	conc. (g/ml)	colony diameter(cm)		%inhibition	sporulation (in grades)
Leaf		Mean	S.E		
	0.0	7.0	0.09	0	++
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem					
	0.0	7.0	0.09	0.0	++
	0.025	3.6	0.03	48.0	-
	0.033	2.9	0.08	58.6	-
	0.05	2.5	0.05	64.6	-
	0.1	1.3	0.04	80.9	-
	0.2	0.4	0.10	95.0	-
Root					
	0.0	7.0	0.09	0.0	++
	0.025	4.4	0.16	37.1	+
	0.033	3.9	0.09	44.3	-
	0.05	3.2	0.08	54.3	-
	0.1	2.3	0.01	67.1	-
	0.2	1.2	0.05	82.9	-

Table 1(d). Effect of *Solanum nigrum* extracts from one month old plants on mycelial growth and sporulation of *Colletotrichum lindemuthianum.*

Extracts	conc. (g/ml)	colony diameter(cm)		%inhibition	sporulation (in grades)
Leaf		Mean	S.E		
	0.0	4.8	0.05	0	+
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem					
	0.0	4.8	0.05	0.0	+
	0.025	2.3	0.12	52.1	-
	0.033	1.8	0.06	62.5	-
	0.05	1.5	0.05	69.8	-
	0.1	1.1	0.03	76.5	-
	0.2	0.4	0.13	92.7	-
Root					
	0.0	4.8	0.05	0.0	+
	0.025	2.9	0.03	39.6	-
	0.033	2.4	0.08	50.0	-
	0.05	2.1	0.09	56.3	-
	0.1	1.5	0.03	68.1	-
	0.2	0.6	0.13	87.5	-

S.E - Standard Error

Table 2(a). Effect of *Solanum nigrum* extracts from two months old plants on mycelial growth and sporulation of *Colletotrichum coffeanum*.

Extracts	conc. (g/ml)	colony diameter(cm)		%inhibition	sporulation (in grades)
Leaf		Mean	S.E		
	0.0	7.7	0.09	0	++
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem[1]					
	0.0	7.7	0.09	0.0	++
	0.025	3.9	0.05	49.4	-
	0.033	3.6	0.09	53.2	-
	0.05	3.0	0.11	61.0	-
	0.1	1.6	0.05	79.2	-
	0.2	0.6	0.04	92.0	-
Stem[2]					
	0.0	7.7	0.09	0.0	++
	0.025	4.0	0.12	48.1	-
	0.033	3.4	0.03	55.8	-
	0.05	2.6	0.20	65.8	-
	0.1	1.7	0.07	77.9	-
	0.2	0.7	0.05	90.9	-
Root					
	0.0	7.7	0.09	0.0	++
	0.025	4.3	0.07	44.2	-
	0.033	3.9	0.07	49.4	-
	0.05	3.4	0.09	55.8	-
	0.1	2.9	0.07	62.3	-
	0.2	1.8	0.07	76.6	-

Stem[1] : Young part of stem
Stem[2] : Older part of stem
S.E : Standard Error

Table 2(b). Effects of *Solanum nigrum* extracts from two months old plants on mycelial growth and sporulation of *Colletotrichum gloeosporioides.*

Extract	conc. (g/ml)	colony diameter(cm)		%inhibition	sporulation (in grades)
Leaf		Mean	S.E		
	0.0	6.3	0.03	0	++
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem[1]					
	0.0	6.3	0.03	0.0	++
	0.025	3.9	0.07	38.4	+
	0.033	3.4	0.07	45.7	-
	0.05	2.7	0.05	57.3	-
	0.1	1.8	0.06	72.4	-
	0.2	0.7	0.03	89.7	-
Stem[2]					
	0.0	6.3	0.03	0.0	++
	0.025	4.0	0.04	36.8	+
	0.033	3.4	0.03	46.6	-
	0.05	2.7	0.00	57.3	-
	0.1	1.7	0.04	73.1	-
	0.2	0.7	0.08	88.1	-
Root					
	0.0	6.3	0.03	0.0	++
	0.025	4.0	0.14	36.8	+
	0.033	3.5	0.10	44.7	-
	0.05	3.1	0.07	51.0	-
	0.1	2.6	0.06	59.8	-
	0.2	1.5	0.11	76.3	-

Stem[1] : Young part of stem
Stem[2] : Older part of stem
S.E : Standard Error

Table 2 (c).Effects of *Solanum nigrum* extracts from two months old plants on mycelial growth and sporulation of *Colletotrichum musae.*

Extract	Conc. g/ml	Colony diameter(cm)		%inhibition	Sporulation (in grades)
Leaf		Mean	S.E		
	0.0	7.1	0.09	0	++
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem[1]					
	0.0	7.1	0.09	0.0	++
	0.025	3.9	0.04	45.1	-
	0.033	3.2	0.04	54.9	-
	0.05	2.7	0.07	62.0	-
	0.1	1.7	0.07	76.1	-
	0.2	0.7	0.05	90.1	-
Stem[2]					
	0.0	7.1	0.09	0.0	++
	0.025	4.0	0.08	43.7	-
	0.033	3.1	0.06	56.3	-
	0.05	2.6	0.03	63.0	-
	0.1	1.9	0.03	73.5	-
	0.2	0.8	0.07	88.7	-
Root					
	0.0	7.1	0.09	0.0	++
	0.025	4.3	0.10	39.4	+
	0.033	4.0	0.09	43.7	-
	0.05	3.3	0.14	53.5	-
	0.1	2.6	0.03	62.9	-
	0.2	1.6	0.10	77.5	-

Stem[1] : Young part of stem
Stem[2] : Older part of stem
S.E : Standard Error

Table 2(d). Effects of *Solanum nigrum* extracts from two months old plants on mycelial growth and sporulation of *Colletotrichum lindemuthianum.*

Extract	conc. (g/ml)	colony diameter(cm)		%inhibition	sporulation (in grades)
Leaf		Mean	S.E		
	0.0	4.9	0.05	0	+
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem[1]					
	0.0	4.9	0.05	0.0	+
	0.025	2.5	0.03	50.0	-
	0.033	2.3	0.15	53.1	-
	0.5	1.7	0.03	65.7	-
	0.1	1.0	0.07	79.6	-
	0.2	0.3	0.04	93.9	-
Stem[2]					
	0.0	4.9	0.05	0.0	+
	0.025	2.5	0.15	49.0	-
	0.033	2.5	0.12	49.0	-
	0.05	1.7	0.03	65.7	-
	0.1	1.1	0.05	77.6	-
	0.2	0.3	0.03	94.9	-
Root					
	0.0	4.9	0.05	0.0	+
	0.025	2.7	0.11	44.9	-
	0.033	2.5	0.05	49.8	-
	0.05	2.1	0.05	57.1	-
	0.1	1.6	0.03	67.8	-
	0.2	0.8	0.04	83.7	-

Stem[1] : Young part of stem
Stem[2] : Older part of stem
S.E : Standard Error

Table 3(a). Effects of *Solanum nigrum* extracts from three months old plants on mycelial growth and sporulation of *Colletotrichum coffeanum*.

Extract	conc. (g/ml)	colony diameter(cm)		%inhibition	sporulation (in grades)
Leaf		Mean	S.E		
	0.0	7.8	0.04	0	++
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem[1]					
	0.0	7.8	0.04	0.0	++
	0.025	3.1	0.04	60.2	-
	0.033	2.4	0.08	69.2	-
	0.05	2.2	0.07	72.4	-
	0.1	1.4	0.04	82.2	-
	0.2	0.2	0.33	97.4	-
Stem[2]					
	0.0	7.8	0.04	0.0	++
	0.025	3.2	0.20	58.9	-
	0.033	2.5	0.05	68.4	-
	0.05	2.3	0.02	70.0	-
	0.1	1.4	0.05	82.3	-
	0.2	0.2	0.08	97.4	-
Root					
	0.0	7.8	0.04	0.0	++
	0.025	4.2	0.04	46.1	-
	0.033	3.7	0.08	52.5	-
	0.05	2.8	0.10	64.1	-
	0.1	1.7	0.04	78.4	-
	0.2	0.5	0.07	93.6	-

Stem[1] : Young part of stem
Stem[2] : Older part of stem
S.E : Standard Error

Table 3(b). Effects of *Solanum nigrum* extracts from three months old plants on mycelial growth and sporulation of *Colletotrichum gloeosporioides*.

Extract	conc. (g/ml)	colony diameter(cm)		%inhibition	sporulation (in grades)
Leaf		Mean	S.E		
	0.0	6.4	0.09	0	++
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem[1]					
	0.0	6.4	0.09	0.0	++
	0.025	2.9	0.03	54.2	-
	0.033	2.3	0.05	64.1	-
	0.05	2.3	0.10	64.1	-
	0.1	1.2	0.09	81.3	-
	0.2	0.3	0.13	95.3	-
Stem[2]					
	0.0	6.4	0.09	0.0	++
	0.025	3.0	0.14	53.1	-
	0.033	2.4	0.05	62.5	-
	0.05	2.3	0.05	64.1	-
	0.1	1.2	0.03	81.6	-
	0.2	0.2	0.08	96.9	-
Root					
	0.0	6.4	0.09	0.0	++
	0.025	3.7	0.09	42.2	+
	0.033	3.2	0.06	50.0	-
	0.05	2.5	0.03	61.1	-
	0.1	1.5	0.02	76.6	-
	0.2	0.3	0.06	95.2	-

Stem[1] : Young part of stem
Stem[2] : Older part of stem
S.E : Standard Error

Table 3(c). Effects of *Solanum nigrum* extracts from three months old plants on mycelial growth and sporulation of *Colletotrichum musae*.

Extract	conc. (g/ml)	colony diameter(cm)		%inhibition	sporulation (in grades)
Leaf		Mean	S.E		
	0.0	7.2	0.08	0	++
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem[1]					
	0.0	7.2	0.08	0.0	++
	0.25	3.3	0.04	54.2	-
	0.033	2.5	0.07	65.3	-
	0.05	2.2	0.11	70.0	-
	0.1	1.3	0.03	81.5	-
	0.2	0.2	0.14	97.2	-
Stem[2]					
	0.0	7.2	0.08	0.0	++
	0.025	3.4	0.13	52.8	-
	0.033	2.5	0.87	65.3	-
	0.05	2.0	0.09	71.8	-
	0.1	1.3	0.03	81.5	-
	0.2	0.2	0.14	97.2	-
Root					
	0.0	7.2	0.08	0.0	++
	0.025	4.0	0.17	44.4	-
	0.033	3.5	0.07	51.4	-
	0.05	2.7	0.09	62.5	-
	0.1	1.5	0.10	79.2	-
	0.2	0.3	0.08	95.8	-

Stem[1] : Young part of stem
Stem[2] : Older part of stem
S.E : Standard Error

Table 3(d). Effects of *Solanum nigrum* extracts from three months old plants on mycelial growth and sporulation of *Colletotrichum lindemuthianum*.

Extract	conc. (g/ml)	colony diameter(cm)		%inhibition	sporulation (in grades)
Leaf		Mean	S.E		
	0.0	5.0	0.08	0	+
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem[1]					
	0.0	5.0	0.08	0.0	+
	0.025	1.8	0.04	64.2	-
	0.033	1.4	0.08	71.4	-
	0.05	1.0	0.08	79.4	-
	0.1	1.0	0.09	80.4	-
	0.2	0.1	0.10	98.0	-
Stem[2]					
	0.0	5.0	0.08	0.0	+
	0.025	1.9	0.09	62.0	-
	0.033	1.4	0.05	72.0	-
	0.05	1.1	0.03	77.4	-
	0.1	0.9	0.08	82.4	-
	0.2	0.0	0.00	100.0	-
Root					
	0.0	5.0	0.08	0.0	+
	0.025	2.5	0.07	50.0	-
	0.033	2.2	0.02	56.0	-
	0.05	1.8	0.06	64.4	-
	0.1	0.9	0.09	82.2	-
	0.2	0.1	0.12	98.0	-

Stem[1] : Young part of stem
Stem[2] : Older part of stem
S.E : Standard Error

Table 4(a). Effects of *Solanum nigrum* extracts from four months old plants on mycelial growth and sporulation of *Colletotrichum coffeanum.*

Extract	conc. (g/ml)	colony diameter(cm)		%inhibition	sporulation (in grades)
		Mean	S.E		
Leaf					
	0.0	7.6	0.11	0	++
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem[1]					
	0.0	7.6	0.11	0.0	++
	0.025	4.3	0.12	43.6	+
	0.033	3.9	0.04	48.9	-
	0.05	3.0	0.03	60.9	-
	0.1	1.9	0.03	74.7	-
	0.2	0.9	0.50	88.2	-
Stem[2]					
	0.0	7.6	0.11	0.0	++
	0.025	4.3	0.02	42.3	+
	0.033	3.8	0.09	50.2	-
	0.05	3.0	0.00	60.7	-
	0.1	2.2	0.03	70.8	-
	0.2	0.9	0.19	88.5	-
Root					
	0.0	7.6	0.11	0.0	++
	0.025	4.2	0.07	45.0	+
	0.033	4.1	0.10	46.3	-
	0.05	3.7	0.04	51.5	-
	0.1	2.4	0.02	68.0	-
	0.2	1.3	0.04	83.0	-
Fruit[1]					
	0.0	7.8	0.08	0.0	++
	0.025	1.2	0.06	84.6	-
	0.033	0.8	0.08	89.7	-
	0.05	0.7	0.07	91.2	-
	0.1	0.0	0.0	100.0	-
	0.2	0.0	0.0	100.0	-
Fruit[2]					
	0.0	7.8	0.08	0.0	++
	0.025	3.0	0.18	61.5	-
	0.033	2.4	0.02	68.7	-
	0.05	2.2	0.08	71.8	-
	0.1	1.4	0.07	82.1	-
	0.2	0.2	0.14	97.4	-
Fruit[3]					
	0.0	7.8	0.08	0.0	++
	0.025	5.3	0.09	32.2	+
	0.033	4.8	0.10	38.5	+
	0.05	4.4	0.08	43.6	-
	0.1	3.3	0.03	58.3	-
	0.2	2.1	0.03	72.8	-

Stem[1] : Young part of stem
Stem[2] : Older part of stem
Fruit[1] : Imature fruit
Fruit[2] : Mature fruit
Fruit[3] : Ripe fruit
S.E : Standard Error

Table 4(b). Effects of *Solanum nigrum* extracts from four months old plants on mycelial growth and sporulation of *Colletotrichum gloeosporioides*.

Extract	conc. (g/ml)	colony diameter(cm)		%inhibition	sporulation (in grades)
Leaf		Mean	S.E		
	0.0	6.4	0.05	0	++
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem[1]					
	0.0	6.4	0.05	0.0	++
	0.025	4.3	0.11	31.8	+
	0.033	3.9	0.02	38.9	+
	0.05	3.0	0.07	52.8	-
	0.1	2.3	0.02	63.8	-
	0.2	0.9	0.29	86.8	-
Stem[2]					
	0.0	6.4	0.05	0.0	++
	0.025	4.3	0.04	32.3	+
	0.033	3.6	0.07	43.3	+
	0.05	3.0	0.04	52.8	-
	0.1	2.3	0.05	63.8	-
	0.2	0.8	0.04	87.4	-
Root					
	0.0	6.4	0.05	0.0	++
	0.025	3.9	0.07	38.6	+
	0.033	3.9	0.06	39.4	+
	0.05	3.1	0.04	51.2	-
	0.1	2.4	0.05	62.8	-
	0.2	1.2	0.07	81.1	-
Fruit[1]					
	0.0	6.3	0.09	0.0	++
	0.025	1.1	0.06	81.9	-
	0.033	0.8	0.05	88.1	-
	0.05	0.5	0.04	91.6	-
	0.1	0.0	0.00	100.0	-
	0.2	0.0	0.00	100.0	-
Fruit[2]					
	0.0	6.3	0.09	0.0	++
	0.025	2.8	0.13	55.5	-
	0.033	2.2	0.02	65.1	-
	0.05	2.2	0.11	65.9	-
	0.1	1.3	0.07	79.4	-
	0.2	0.3	0.04	95.2	-
Fruit[3]					
	0.0	6.3	0.09	0.0	++
	0.025	6.0	0.09	20.6	++
	0.033	4.5	0.07	28.6	+
	0.05	3.9	0.06	38.1	+
	0.1	2.8	0.07	55.5	-
	0.2	1.8	0.03	71.8	-

Stem[1] : Young part of stem
Stem[2] : Older part of stem
Fruit[1] : Imature fruit
Fruit[2] : Mature fruit
Fruit[3] : Ripe fruit
S.E : Standard Error

Table 4(c). Effects of *Solanum nigrum* extracts from four months old plants on mycelial growth and sporulation of *Colletotrichum musae*.

Extract	conc. (g/ml)	colony diameter(cm)		%inhibition	sporulation (in grades)
		Mean	S.E		
Leaf					
	0.0	7.0	0.04	0	++
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem[1]					
	0.0	7.0	0.04	0.0	++
	0.025	4.0	0.07	42.9	-
	0.033	3.4	0.07	51.4	-
	0.05	3.1	0.05	56.0	-
	0.1	1.8	0.03	73.9	-
	0.2	0.8	0.08	88.6	-
Stem[2]					
	0.0	7.0	0.04	0.0	++
	0.025	3.8	0.09	45.7	-
	0.033	3.6	0.03	48.1	-
	0.05	3.2	0.03	54.6	-
	0.1	2.0	0.09	71.4	-
	0.2	0.8	0.16	88.9	-
Root					
	0.0	7.0	0.04	0.0	++
	0.025	4.2	0.11	40.0	+
	0.033	3.9	0.14	44.3	-
	0.05	3.6	0.09	49.3	-
	0.1	2.3	0.03	66.7	-
	0.2	1.3	0.10	81.4	-
Fruit[1]					
	0.0	7.1	0.11	0.0	++
	0.025	1.1	0.08	84.6	-
	0.033	0.7	0.04	90.9	-
	0.05	0.7	0.04	89.8	-
	0.1	0.0	0.00	100.0	-
	0.2	0.0	0.00	100.0	-
Fruit[2]					
	0.0	7.1	0.13	0.0	++
	0.025	2.8	0.04	60.6	-
	0.033	2.3	0.07	67.6	-
	0.05	2.3	0.02	67.0	-
	0.1	1.4	0.03	79.9	-
	0.2	0.3	0.18	95.8	-
Fruit[3]					
	0.0	7.1	0.11	0.0	++
	0.025	5.0	0.07	29.9	++
	0.033	4.7	0.08	33.9	+
	0.05	4.1	0.07	42.5	-
	0.1	3.0	0.03	57.7	-
	0.2	1.9	0.02	73.6	-

Stem[1] : Young part of stem
Stem[2] : Older part of stem
Fruit[1] : Imature fruit
Fruit[2] : Mature fruit
Fruit[3] : Ripe fruit
S.E : Standard Error

Table 4(d). Effects of *Solanum nigrum* extracts from four months old plants on mycelial growth and sporulation of *Colletotrichum lindemuthianum*.

Extract	conc. (g/ml)	colony diameter(cm)		%inhibition	sporulation (in grades)
		Mean	S.E		
Leaf					
	0.0	4.9	0.04	0	+
	0.025	0.0	0.0	100	-
	0.033	0.0	0.0	100	-
	0.05	0.0	0.0	100	-
	0.1	0.0	0.0	100	-
	0.2	0.0	0.0	100	-
Stem[1]					
	0.0	4.9	0.04	0.0	+
	0.025	2.7	0.04	44.9	-
	0.033	2.4	0.05	51.0	-
	0.05	1.7	0.04	65.3	-
	0.1	1.3	0.06	73.5	-
	0.2	0.4	0.09	91.8	-
Stem[2]					
	0.0	4.9	0.04	0.0	+
	0.025	2.9	0.07	41.8	-
	0.033	2.3	0.07	53.1	-
	0.05	2.0	0.06	59.2	-
	0.1	1.3	0.03	72.9	-
	0.2	0.2	0.14	96.3	-
Root					
	0.0	4.9	0.04	0.0	+
	0.025	2.7	0.01	45.3	+
	0.033	2.5	0.05	49.8	-
	0.05	2.1	0.09	47.1	-
	0.1	1.8	0.02	62.4	-
	0.2	0.7	0.08	85.7	-
Fruit[1]					
	0.0	4.9	0.12	0.0	+
	0.025	0.6	0.05	87.8	-
	0.033	0.5	0.03	89.2	-
	0.05	0.3	0.05	94.9	-
	0.1	0.0	0.00	100.0	-
	0.2	0.0	0.00	100.0	-
Fruit[2]					
	0.0	4.9	0.12	0.0	+
	0.025	1.8	0.03	62.7	-
	0.033	1.6	0.08	67.3	-
	0.05	1.4	0.02	71.4	-
	0.1	0.8	0.04	82.9	-
	0.2	0.0	0.00	100.0	-
Fruit[3]					
	0.0	4.9	0.12	0.0	+
	0.025	3.3	0.14	32.7	+
	0.033	3.3	0.05	32.1	+
	0.05	2.5	0.08	49.0	-
	0.1	2.0	0.04	59.2	-
	0.2	1.1	0.03	76.6	-

Stem[1] : Young part of stem
Stem[2] : Older part of stem
Fruit[1] : Immature fruit
Fruit[2] : Mature fruit
Fruit[3] : Ripe fruit
S.E : Standard Error

Table 5. Effect of *Solanum nigrum* extracts on growth rates (cm/day) of the species of *Colletotrichum* on PDA.

Plant age	Extract	Conc. (g/ml)	Avarage growth rate in (cm/day)			
			A	B	C	D
1	Leaf	0.0	0.96	0.78	0.88	0.6
		0.025	0.0	0.0	0.0	0.0
		0.033	0.0	0.0	0.0	0.0
		0.05	0.0	0.0	0.0	0.0
		0.1	0.0	0.0	0.0	0.0
		0.2	0.0	0.0	0.0	0.0
	Stem	0.0	0.96	0.78	0.88	0.6
		0.025	0.45	0.43	0.45	0.29
		0.033	0.39	0.36	0.36	0.23
		0.05	0.33	0.30	0.31	0.18
		0.1	0.20	0.20	0.17	0.14
		0.2	0.08	0.06	0.04	0.04
	Root	0.0	0.96	0.78	0.88	0.6
		0.025	0.56	0.55	0.55	0.36
		0.033	0.50	0.49	0.49	0.30
		0.05	0.42	0.39	0.40	0.26
		0.1	0.31	0.29	0.29	0.19
		0.2	0.09	0.06	0.05	0.08
2	Leaf	0.0	0.96	0.79	0.89	0.61
		0.025	0.0	0.0	0.0	0.0
		0.033	0.0	0.0	0.0	0.0
		0.05	0.0	0.0	0.0	0.0
		0.1	0.0	0.0	0.0	0.0
		0.2	0.0	0.0	0.0	0.0
	Stem[1]	0.0	0.96	0.79	0.89	0.61
		0.025	0.48	0.49	0.49	0.31
		0.033	0.45	0.43	0.40	0.29
		0.05	0.38	0.34	0.34	0.21
		0.1	0.20	0.22	0.21	0.13
		0.2	0.08	0.08	0.9	0.04
	Stem[2]	0.0	0.96	0.79	0.89	0.61
		0.025	0.50	0.50	0.50	0.31
		0.033	0.43	0.42	0.39	0.31
		0.05	0.33	0.34	0.33	0.21
		0.1	0.21	0.21	0.24	0.14
		0.2	0.09	0.09	0.1	0.03
	Root	0.0	0.96	0.79	0.89	0.61
		0.025	0.54	0.50	0.54	0.34
		0.033	0.49	0.44	0.50	0.31
		0.05	0.43	0.39	0.41	0.26
		0.1	0.36	0.32	0.33	0.20
		0.2	0.23	0.19	0.20	0.10
3	Leaf	0.0	0.97	0.8	0.9	0.63
		0.025	0.0	0.0	0.0	0.0
		0.033	0.0	0.0	0.0	0.0
		0.05	0.0	0.0	0.0	0.0
		0.1	0.0	0.0	0.0	0.0
		0.2	0.0	0.0	0.0	0.0
	Stem[1]	0.0	0.97	0.8	0.9	0.63
		0.025	0.39	0.37	0.41	0.22
		0.033	0.30	0.29	0.31	0.18
		0.05	0.27	0.29	0.27	0.13
		0.1	0.17	0.15	0.17	0.12
		0.2	0.03	0.04	0.03	0.04

	Stem[2]	0.0	0.97	0.8	0.9	0.63
		0.025	0.4	0.38	0.43	0.24
		0.033	0.31	0.30	0.30	0.18
Table 5 continued		0.05	0.29	0.29	0.25	0.14
		0.1	0.17	0.15	0.17	0.11
		0.2	0.03	0.02	0.03	0.0
	Root	0.0	0.97	0.8	0.9	0.63
		0.025	0.53	0.46	0.5	0.31
		0.033	0.46	0.40	0.44	0.28
		0.05	0.35	0.31	0.34	0.22
		0.1	0.21	0.19	0.19	0.11
4	Leaf	0.0	0.95	0.79	0.88	0.61
		0.025	0.0	0.0	0.0	0.0
		0.033	0.0	0.0	0.0	0.0
		0.05	0.0	0.0	0.0	0.0
		0.1	0.0	0.0	0.0	0.0
		0.2	0.0	0.0	0.0	0.0
	Stem[1]	0.0	0.95	0.79	0.88	0.61
		0.025	0.54	0.54	0.50	0.34
		0.033	0.49	0.49	0.43	0.30
		0.05	0.37	0.38	0.39	0.21
		0.1	0.24	0.29	0.23	0.16
		0.2	0.11	0.11	0.1	0.05
	Stem[2]	0.0	0.95	0.79	0.88	0.61
		0.025	0.55	0.54	0.48	0.37
		0.033	0.48	0.45	0.45	0.29
		0.05	0.38	0.34	0.40	0.25
		0.1	0.29	0.29	0.25	0.17
		0.2	0.11	0.10	0.10	0.02
	Root	0.0	0.95	0.79	0.88	0.61
		0.025	0.53	0.49	0.53	0.34
		0.033	0.51	0.48	0.49	0.31
		0.05	0.46	0.39	0.44	0.26
		0.1	0.31	0.30	0.29	0.23
		0.2	0.16	0.15	0.16	0.09
	Fruit[1]	0.0	0.98	0.79	0.89	0.61
		0.025	0.15	0.14	0.14	0.08
		0.033	0.10	0.09	0.08	0.07
		0.05	0.09	0.07	0.09	0.03
		0.1	0.0	0.0	0.0	0.0
		0.2	0.0	0.0	0.0	0.0
	Fruit[2]	0.0	0.98	0.79	0.89	0.61
		0.025	0.38	0.35	0.35	0.23
		0.033	0.31	0.28	0.29	0.20
		0.05	0.28	0.27	0.29	0.18
		0.1	0.18	0.16	0.18	0.11
		0.2	0.03	0.04	0.04	0.0
	Fruit[3]	0.0	0.98	0.79	0.89	0.61
		0.025	0.66	0.63	0.63	0.41
		0.033	0.6	0.56	0.59	0.42
		0.05	0.55	0.49	0.51	0.31
		0.1	0.41	0.35	0.38	0.25
		0.2	0.27	0.22	0.24	0.14

A : *C. coffeanum*
B : *C. gloeosporioides*
C : *C. musae*
D : *C. lindemuthianum*

Stem[1] : Young part of stem
Stem[2] : Older part of stem
Fruit[2] : Mature fruit
Fruit[3] : Ripe fruit
Fruit[1] : Immature fruit

4.2.1 Effects of extracts from different parts of *S. nigrum* on species of *Colletotrichum.*

4.2.1.1 One month old plants

The fungal colony diameters were found to vary for extracts made from different plant parts. In plates treated with leaf extracts there was no mycelial growth. Plates treated with root extracts had larger colony diameters than those treated with stem extracts Fig 1(a-d). Thus extracts from different parts of *S. nigrum* had varying inhibitory effects on the test fungi. Leaf extracts were found to have 100 % inhibition on all the four *Colletotrichum* species (Table 1-4). Stem extracts had a slightly higher percentage inhibition than the root extracts. The differences in inhibition of *Colletotrichum* species by extracts from different plant parts of one month old plants were significant (Table 6a).

4.2.1.2 Two months old plants

Experimental tests with extracts from two months old plants also showed that there was a significant difference in fungal inhibition by extracts from different plant parts Fig. 1(a-d). At this age the stem was subdivided into stem^1 (young part of stem) and stem^2 (the older part of the stem). Hence, there were four plant parts. Fungal colony diameters were found to vary for the different extracts. On plates treated with leaf extracts there was no mycelial growth. The differences in colony diameters on plates treated with stem^1 and stem^2 extracts were negligible. Colony diameters on plates treated with root extracts were larger than those on the plates treated with stem extracts Fig.1(a-d). Thus the extracts had varying inhibitory effects, with leaf extracts being

the most effective and root extracts the least effective (Plate 2a, 2b and 2c). The differences in fungal inhibition by Stem[1] and Stem[2] extracts were negligible. Inhibition by Leaf extracts was significantly different from inhibition by extracts from all other plant parts, as were the root extracts. But the differences in inhibition by stem [1] and stem[2] extracts were not significant (Table 6b).

4.2.1.3 Three months old plants.

Differences in colony diameters on the plates treated with the different extracts from three months old *S. nigrum* plants, were negligible Fig. 1(a-d). Thus there was no significant difference in fungal inhibition by the extracts from different parts of three months old plants. However for the species *Colletotrichum coffeanum*, the plates treated with root extracts had larger colony diameters (Fig. 1a). Hence for the species *C. coffeanum* leaf extracts differed significantly from root extracts. Differences in fungal inhibition by extracts from all other parts were not significant (Table 6c).

4.2.1.4 Four months old plants.

Extracts from four months old plants were made from seven plant parts ; leaves, stem[1], stem[2], root, immature fruits, mature fruits and red ripe fruits. Radial mycelial growth varied among the extracts. The degree of inhibition thus varied among the extracts. Extracts from leaves, immature fruit and mature green fruits inhibited the fungi most. At a concentration level of 0.2 g/ml. leaf and immature fruit extracts had total inhibition on all the four *Colletotrichum* species. With the green mature fruits the percentage inhibition was 97.4 on *C. coffeanum*, 95.2 on *C. gloeosporioides* 95.7 on *C. musae* and 100% on *C. lindemuthianum*. The ripe red fruits had the lowest inhibitory

lindemuthianum. The ripe red fruits had the lowest inhibitory effects with inhibitions of 72.7, 71.7, 73.7 and 77% for *C. coffeanum*, *C. gloeosporioides*, *C. musae* and *C. lindemuthianum* respectively. Thus the differences in fungal inhibition by leaves, immature fruits and mature fruits extracts at a concentration level of 0.2g/ml. were not significant. Mycelial growth inhibition by extracts from ripe fruits were significantly different from inhibitions by extracts from all other parts (Table 6d).

The degree of inhibition by each extract on the four species revealed that the response was variable. With one month old plants the differences in inhibition by leaf and stem extracts on the four species were not significant (Table 6a). However with the root extracts, there was a significant difference amongst the species. *Colletotrichum lindemuthianum* was inhibited more than the other species.

Inhibition of the fungal species by extracts from two months old plants was also variable. There were significant differences in inhibition amongst the species by stem, and root extracts. Of the four species, *Colletotrichum lindemuthianum* was the most inhibited. There was no difference in inhibition amongst the four *Colletotrichum* species by leaf extracts.

The differences in response by the four species when treated with leaf, stem^1, stem^2, and root extracts from three months old plants were not significant (Table 6c). There was no significant differences in response amongst the four fungal species when treated with leaf, stem^1, stem^2, root, immature and mature fruit extracts from four months old plants. However with ripe fruit extracts, the difference in inhibition amongst the four species was significant (Table 6d).

Of the extracts evaluated, 5 out of 18 (27.8%) showed that the differences in response between the four species were significant. Whereas 13 out of 18 (72.2%) cases showed that the differences in response by the different species was not significant.

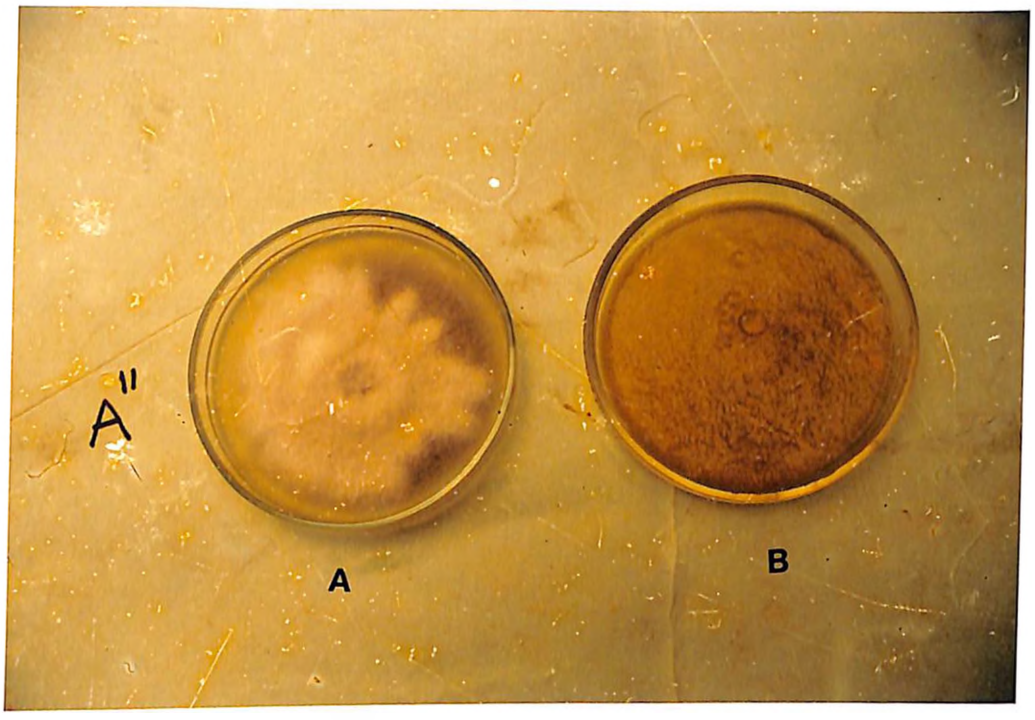

Plate 2(a) Ten day old *Colletotrichum musae* in PDA amended with *Solanum nigrum* leaf extracts from a two months old plant.

A - Control

B - Treated

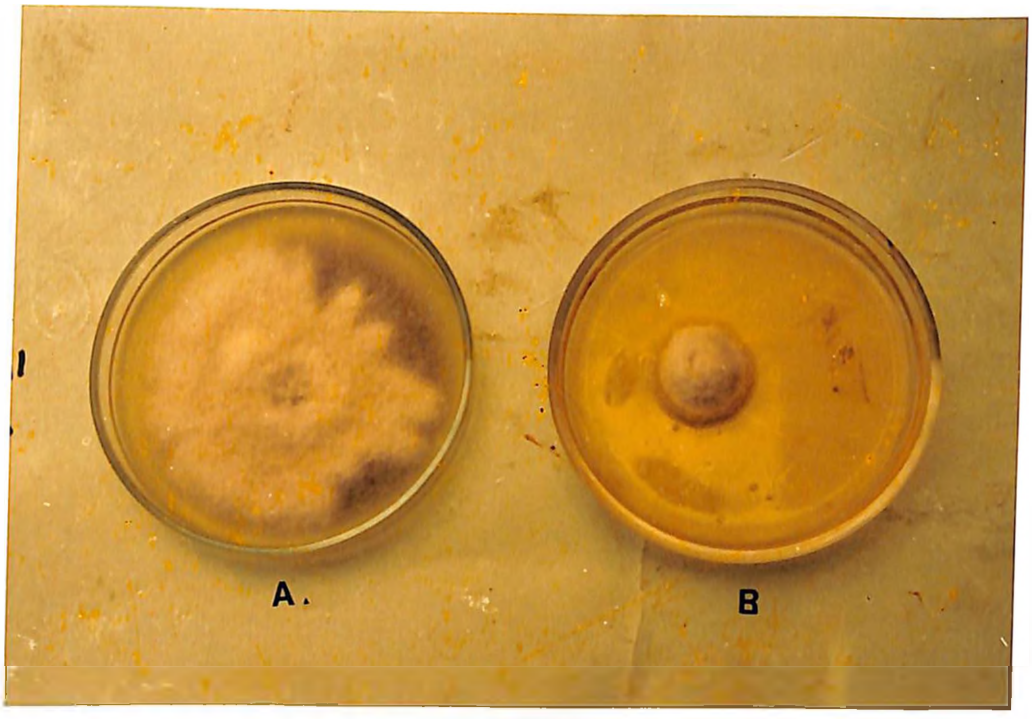

Plate 2(b). Ten day old *Colletotrichum musae* in PDA amended with *Solanum nigrum* stem extracts from a two months old plant at a concentration level of 0.05g/ml.

A - Control

B - Treated

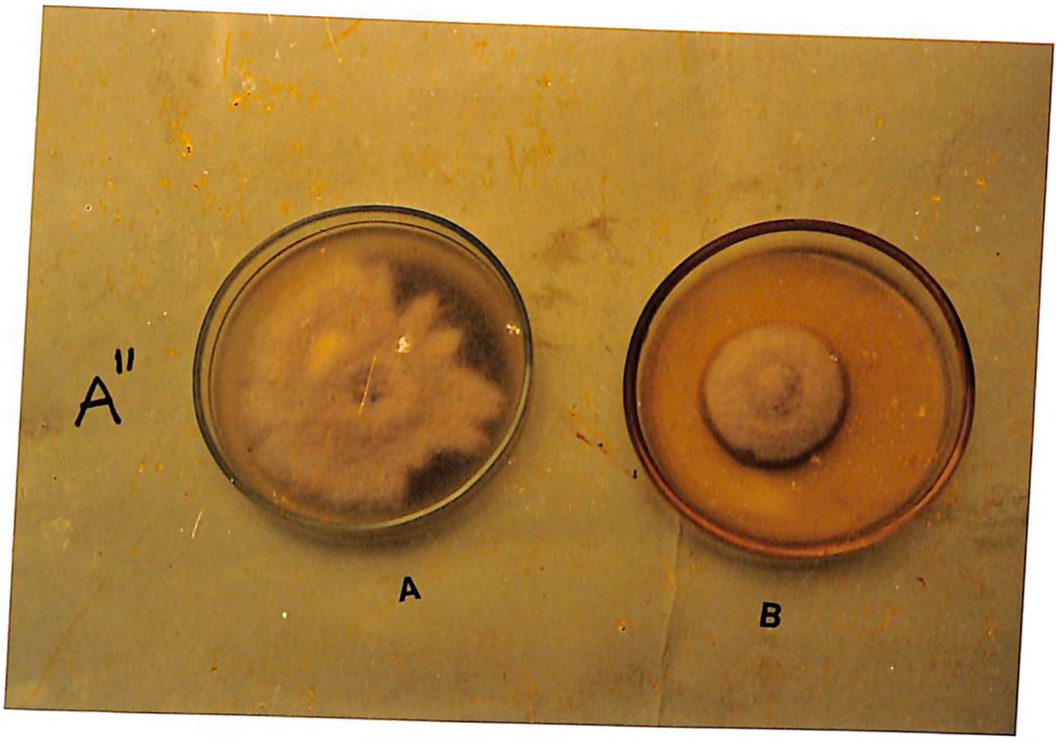

Plate 2(c). Ten day old *Colletotrichum musae* in PDA amended with *S.nigrum* root extracts from a two months old plant at a concentration of 0.05 g/ml.

A - Control

B - Treated

Table: 6. Degree of inhibition[z] by extracts from different plant parts. The values given are group means and the corresponding standard errors (S.E.). n=4 in all cases.

key:-

A: *Colletotrichum coffeanum*

B: *Colletotrichum gloeosporioides*

C: *Colletotrichum musae*

D: *Colletotrichum lindemuthianum*

$$z: \quad \frac{C - T}{C} \times 10$$

Table 6(a). Fungal inhibitions by extracts from one month old plants.

Species	Leaf	Stem	Root	df	F	P
A	10.0 ± 0.0	9.22 0.53	8.05 0.53	2,9	513.0	p<0.05
B	10.0 ± 0.0	9.19 0.113	7.9 0.146	2,9	98.59	p<0.05
C	10.0 ± 0.0	9.50 0.073	9.28 0.116	2,9	124.5	p<0.05
D	10.0 ± 0.0	9.27 0.262	8.75 0.192	2,9	5.84	p<0.05
F	0.0	0.86	7.41			
P	p>0.05	p>0.05	p<0.05			

Table 6(b). Fungal inhibition by extracts from two months old plants.

species	Leaf	stem[1]	Stem[2]	Root	df	F	p
A	10.0 ± 0.0	9.22 0.053	9.09 0.059	7.66 0.092	3,12	257.15	p<0.05
B	10.0 ± 0.0	8.97 0.046	8.89 0.129	7.63 0.171	3,12	79.23	p<0.05
C	10.0 ± 0.0	9.01 0.091	8.87 0.1	7.89 0.15	3,12	69.21	p<0.05
D	10.0 ± 0.0	9.39 0.08	9.59 0.058	8.36 0.08	3,12	107.83	p<0.05
F	0.0	6.94	9.94	6.87			
P	p>0.05	p<0.05	p<0.05	p<0.05			

stem[1] - Young part of stem
stem[2] - Older part of stem

Table 6(c). Fungal inhibition by extracts from three months old plants.

species	Leaf	Stem[1]	Stem[2]	Root	df	F	p
A	10.0 ± 0.0	9.74 0.15	9.74 0.11	9.36 0.09	3,12	6.76	p<0.05
B	10.0 ± 0.0	9.53 0.09	9.69 0.212	9.53 0.2	3,12	1.61	p>0.05
C	10.0 ± 0.0	9.72 0.2	9.72 0.18	9.58 0.18	3,12	1.164	p>0.05
D	10.0 ± 0.0	9.8 0.2	9.8 0.2	10.0 0.0	3,12	0.67	p>0.05
F	0.0	0.397	0.07	3.68			
p	p>0.05	p>0.05	p>0.05	p>0.05			

stem[1] - Young part of stem
stem[2] - Older part of stem

Table 6(d). Fungal inhibition by extracts from four months old plants.

spp	L	S^1	S^2	R	F^1	F^2	F^3	df	F
A	10.0 ± 0.0	8.82 0.12	8.84 0.26	8.29 0.05	10.0 0.0	9.7 0.18	7.28 0.03	6,21	59.64*
B	10.0 ± 0.0	8.66 0.47	8.74 0.06	8.11 0.11	10.0 0.0	9.5 0.07	7.18 0.04	6,21	30.82*
C	10.0 ± 0.0	8.86 0.10	8.89 0.23	8.14 0.15	10.0 0.0	9.57 0.27	7.36 0.28	6,21	43.53*
D	10.0 ± 0.0	9.18 0.19	9.64 0.29	8.57 0.17	10.0 0.0	10.0 0.0	7.70 0.16	6,21	35.75*
F	0.0	0.688	3.25	2.71	0.0	1.668	34.2		
p	+	+	+	+	+	+	*		

\+ = $p>0.05$
* = $p<0.05$
L = Leaf
S^1 = Young part of stem ($Stem^1$)
S^2 = Older part of stem ($Stem^2$)
R = Root
F^1 = Immature fruit ($Fruit^1$)
F^2 = Mature fruit ($Fruit^2$)
F^3 = Ripe fruit ($Fruit^3$)
spp = Species

Fig. 1 (A-D). Effects of *solanum nigrum* extracts at a concentration of 0.2g/ml, from different parts and ages, on the four species of *Colletotrichum.*

NB: Leaf extracts are not presented on the following graphs because the radial mycelial growth (Colony diameter) was zero in all cases.

Fig.1 (a). Effects of *Solanum nigrum* extracts at a concentration of 0.2g/ml, from different parts and ages, on radial mycelial growth of *Colletotrichum coffeanum*.

Fig.1 (b). Effects of *Solanum nigrum* extracts at a concentration of 0.2g/ml, from different parts and ages, on radial mycelial growth of *Colletotrichum gloeosporioides*.

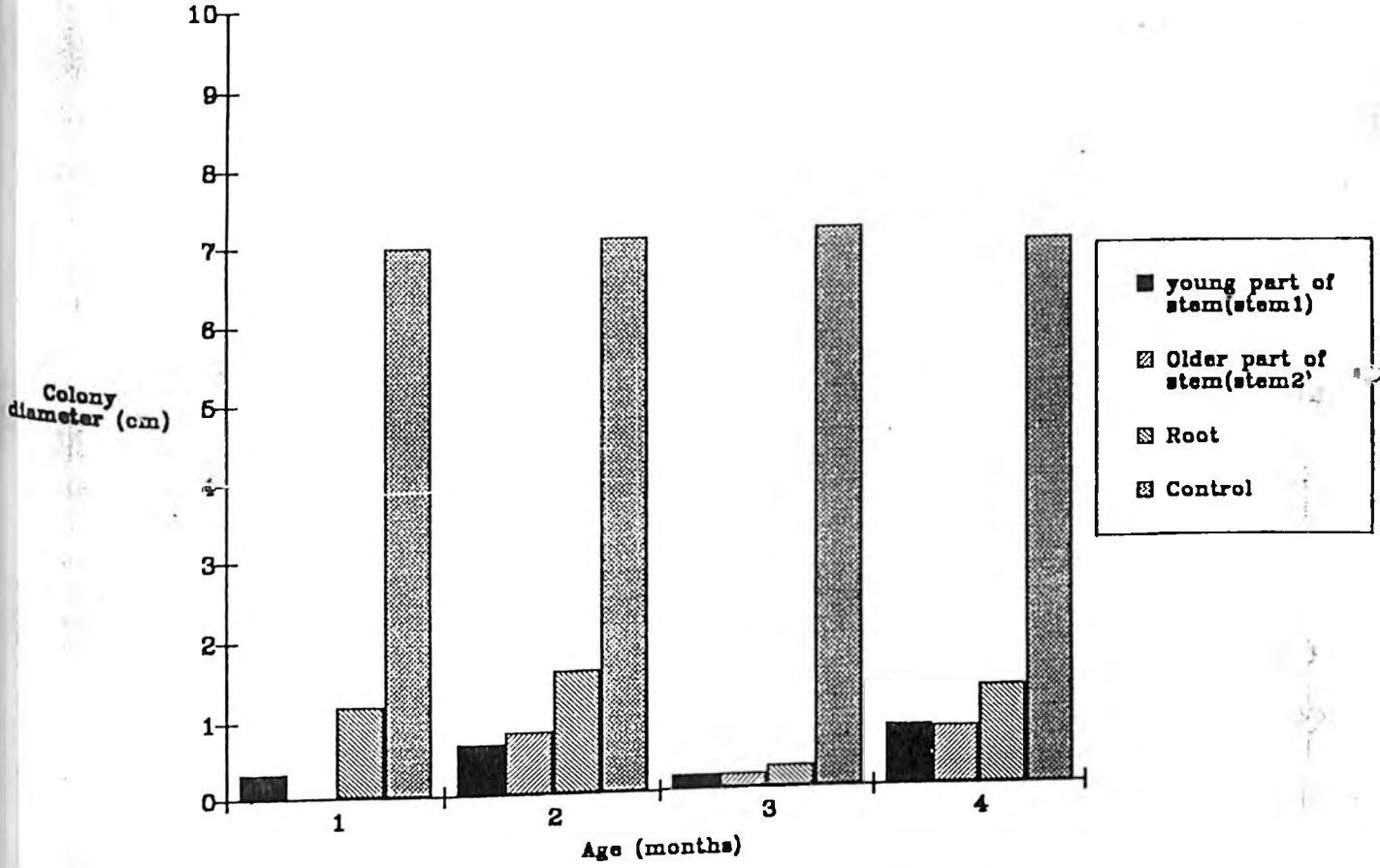

Fig.1 (c). Effects of *Solanum nigrum* extracts at a concentration of 0.2g/ml, from different parts and ages, on radial mycelial growth of *Colletotrichum musae*.

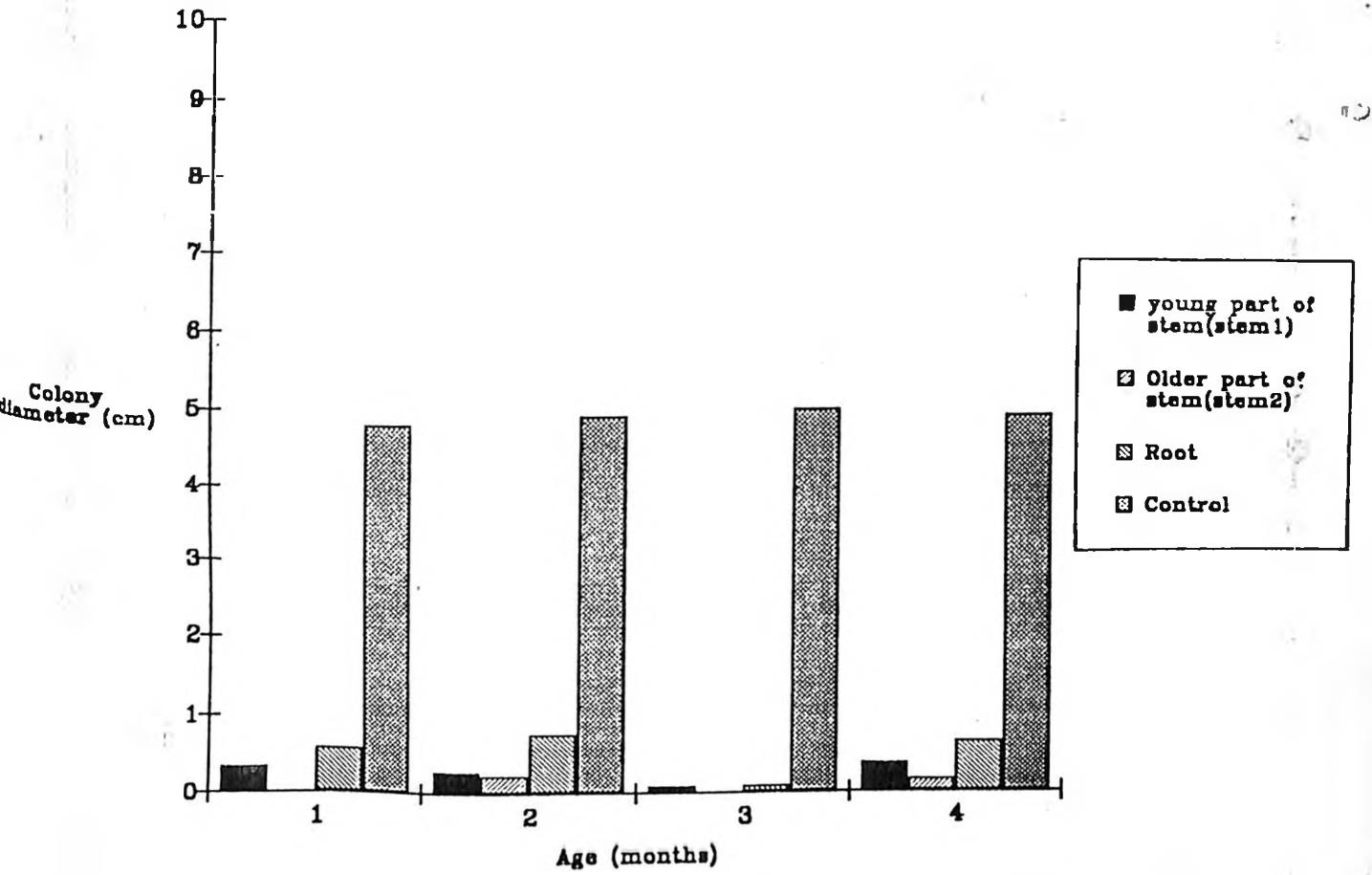

Fig.1 (d). Effects of *Solanum nigrum* extracts at a concentration of 0.2g/ml, from different parts and ages, on radial mycelial growth of *Colletotrichum lindemuthianum*.

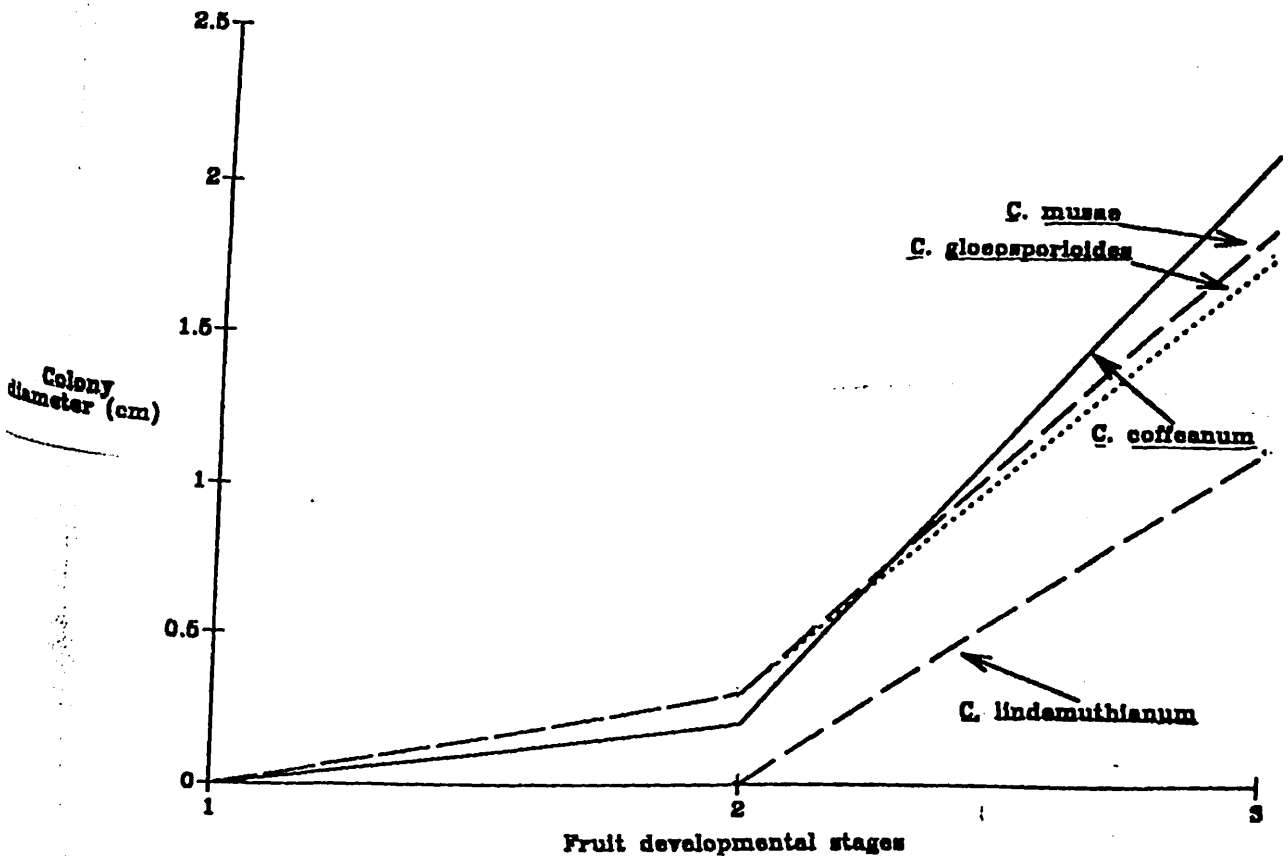

Fig.1 (e). Effects of *Solanum nigrum* extracts at a concentration of 0.2g/ml, from fruits at different developmental stages, on radial mycelial growth of the four species of *Colletotrichum*.

1 - Imature fruits
2 - Mature green fruits
3 - Ripe fruits

4.2.2 Effects of plant age on the degree of inhibition

Extracts from the same parts of the plant at different ages were found to have varying inhibitory effects. This was true for all parts except the leaves. Inhibition by leaf extracts remained 100 per cent at all ages on all the four *Colletotrichum* species (Table 1-4).

4.2.2.1 Leaf extracts

The inhibitive property of the leaf extracts did not change with the age of the plant from four weeks to maturity (16 weeks). Leaf extracts stored at room temperature for five days remained inhibitive. Media amended with leaf extracts and kept at different temperatures, 15, 20, 25, 30 and 40° C for 8 hours before inoculation remained inhibitive. However autoclaving the extracts at 121°C for 20 minutes, completely destroyed the inhibitive property. The radial mycelial growth and sporulation in plates treated with autoclaved extracts was as good as that on the control plates. There was no difference in inhibitory effects by extracts from the youngest leaves and the oldest leaves (Plate 6).

4.2.2.2 Young part of stem (Stem[1]) extracts

The inhibitive property of stem extracts changed with the age of the plant. Thus there was a significant difference in fungal inhibition by stem [1] extracts from different ages of *S. nigrum*. However, this was variable amongst the fungal species. There was a significant difference in fungal inhibition by stem[1] extracts at different *S. nigrum* ages for *C. coffeanum*, *C. musae*, and *C. gloeosporioides* where extracts from three months old

plants had the best inhibition. However for *C. lindemuthianum* the differences in inhibition by stem[1] extracts at different ages was not significant (Table 7a).

4.2.2.3 Older part of stem (Stem [2]) extracts

There was a significant difference in fungal inhibition by stem[2] extracts from different *S. nigrum* ages. For *C. coffeanum, C. gloeosporioides* and *C. musae* extracts from three months old plants had higher inhibitory effects than those from other ages. This difference was significant (Table 7b). For *C. lindemuthianum* the differences in inhibition by stem[2] extracts from plants of different ages were not significant (Table 7b).

4.2.2.4 Root extracts

Roots were not subdivided into parts. There was a significant difference in fungal inhibition by root extracts from plants at different ages. This was true for all the four species of *Colletotrichum.* Extracts from three months old plants gave the best inhibitory effects for all fungi (fig. 1a-d, Table 7c).

4.2.2.5 Fruit extracts

Fruits of different developmental stages were compared and were categorized as; green immature (f1), mature unripe fruits (f2) and ripe red mature fruits (f3) (Plate 3a). There was variation in fungal inhibition by fruit extracts at different stages of development (Plate 3b). Green immature fruits had the highest inhibitory properties and the red ripe fruits had the

lowest (Fig. 1e). However the differences in fungal inhibition between the green immature fruits and the green mature fruits were negligible (Table 7d). Thus fungal inhibition by *Solanum nigrum* stem[1], stem[2] and root extracts vary with plant age and inhibition by fruit extracts vary with developmental stages. Generally the differences in inhibition amongst the four species of *Colletotrichum* were not significant.

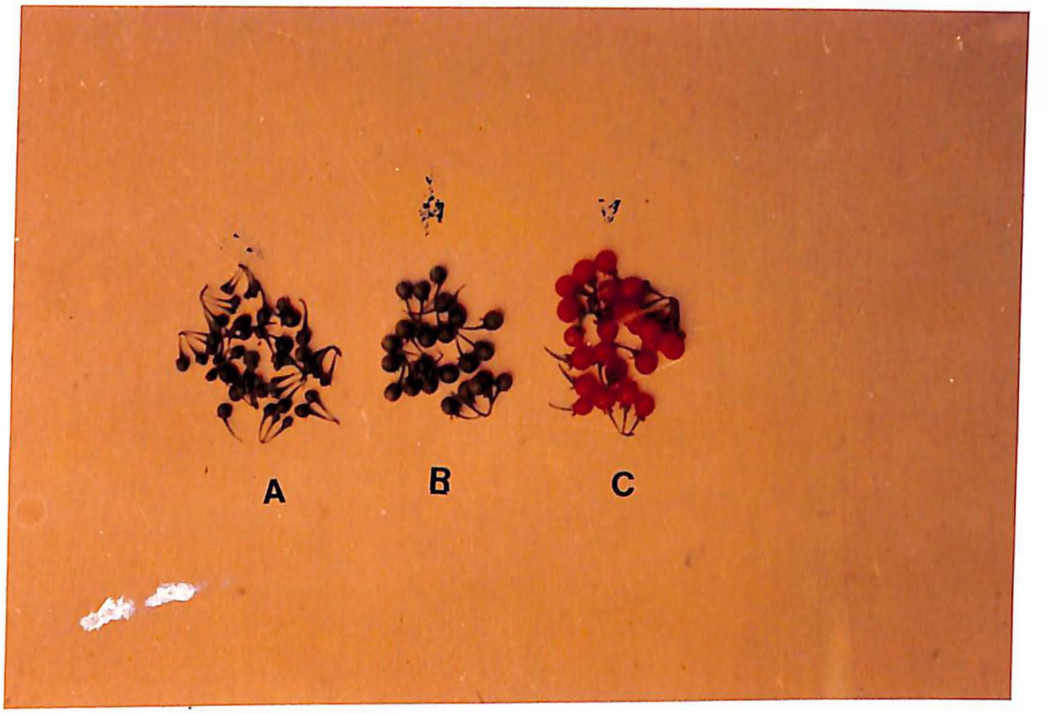

Plate 3(a). *Solanum nigrum* fruits at different stages of development.

A - Imature fruits

B - Mature green fruits

C - Ripe fruits

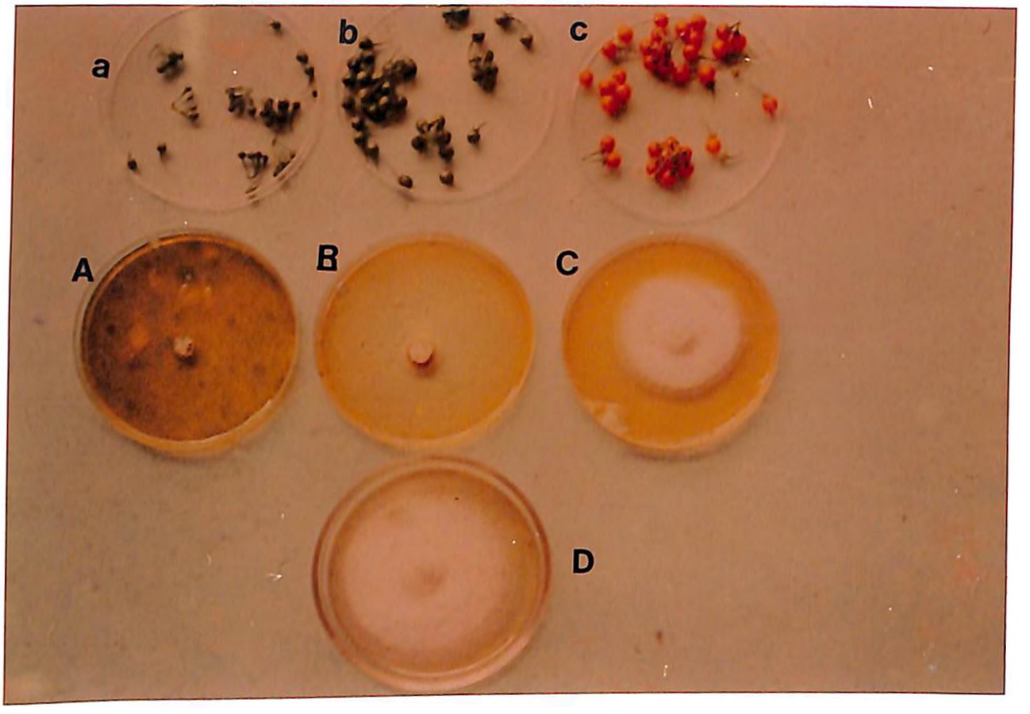

plate 3(b). *Solanum nigrum* fruits at different stages of development and seven day old cultures of *Colletotrichum coffeanum* in PDA amended with fruit extracts at different stages of development at a concentration level of 0.1 g/ml.

A = Green imature fruits.

B = Mature unripe fruits.

C = Red ripe fruits.

D = Control.

Table 7. Inhibition of *Colletotrichum* species by *Solanum nigrum* plant extracts at different ages. Values given are group means and the corresponding standard errors. n=4 in all cases.

Table 7a. Young part of stem (Stem[1]) extracts.

Species	Age (months) 1	2	3	4	df	F	p
A	9.22	9.22	9.74	8.82	3,12	13.25	p<0.05
	± 0.05	0.05	0.15	0.12			
B	9.19	8.97	9.61	8.66	3,12	11.97	p<0.05
	± 0.11	0.05	0.19	0.47			
C	9.49	9.01	9.72	8.86	3,12	10.36	p<0.05
	± 0.07	0.09	0.2	0.10			
D	9.27	9.39	9.8	9.18	3,12	1.98	p>0.05
	± 0.26	0.08	0.2	0.18			

Table 7(b). Older part of stem (stem[2]) extracts.

Species	Age (months) 1	2	3	4	df	F	p
A	-	9.09	9.74	8.85	2,9	7.79	p<0.05
	±	0.06	0.10	0.25			
B	-	8.89	9.69	8.74	2,9	11.85	p<0.05
	±	0.13	0.21	0.06			
C	-	8.87	9.72	8.89	2,9	7.30	p<0.05
	±	0.09	0.19	0.23			
D	-	9.49	9.8	9.64	2,9	0.56	p>0.05
	±	0.06	0.2	0.29			

Table 7(c). Root extracts.

Species	Age (months) 1	2	3	4	df	F	p
A	8.05	7.66	9.36	8.29	3,12	95.18	p<0.05
±	0.05	0.09	0.09	0.05			
B	7.89	7.63	9.53	8.11	3,12	28.04	p<0.05
±	0.14	0.17	0.2	0.1			
C	8.28	7.89	9.58	8.14	3,12	25.42	p<0.05
±	0.12	0.15	0.18	0.14			
D	8.75	8.37	10.0	8.36	3,12	36.18	p<0.05
±	0.19	0.08	0.0	0.2			

Table 7(d). Fruit extracts.

Species	F^1	F^2	F^3	df	F	p
A	10.0	9.74	7.28	2,9	199.4	p<0.05
±	0.0	0.18	0.03			
B	10.0	9.52	7.18	2,9	1164.1	p<0.05
±	0.0	0.07	0.04			
C	10.0	9.58	7.36	2,9	80.56	p<0.05
±	0.0	0.27	0.03			
D	10.0	10.0	7.66	2,9	3885.4	p<0.05
±	0.0	0.0	0.04			

F^1 : Immature fruits
F^2 : Mature fruits
F^3 : Ripe fruits

4.2.3 Effects of dilution of *Solanum nigrum* extracts upon the inhibitive property

Percentage inhibition was found to increase with increasing extract concentration. Thus higher concentrations inhibited fungal mycelial growth more than the lower concentrations (Plate 4a, 4b and fig. 2-6). However this was not true for the leaf extracts. Dilution of leaf extracts up to the level tested in the study had no effect on the inhibitive property (plate 5a and 5b).

Leaf extracts proved to be fungicidal in that no mycelial growth was observed on the test fungi even after the innoculum plugs were transferred to extract free PDA plates and incubated for ten days. This however was also dependent on concentrations. Mycelial plugs transferred from plates that had been treated with lower concentration of the extracts were still viable but the growth was retarded. Mycelial plugs transferred from plates treated with higher extract concentrations, completely lost viability.

There were significant differences in fungal inhibition by stem, root, mature fruits and ripe fruit extracts, at different concetrations (Table 8a and b). Higher concetrations were superior to lower ones in terms of fungal inhibition (fig. 2-6).

Differences in fungal inhibition by imature fruit extracts at concetrations of 0.1g/ml. and 0.2g/ml. were not significant. This was true for all the four species of *Colletotrichum* (Table 4).

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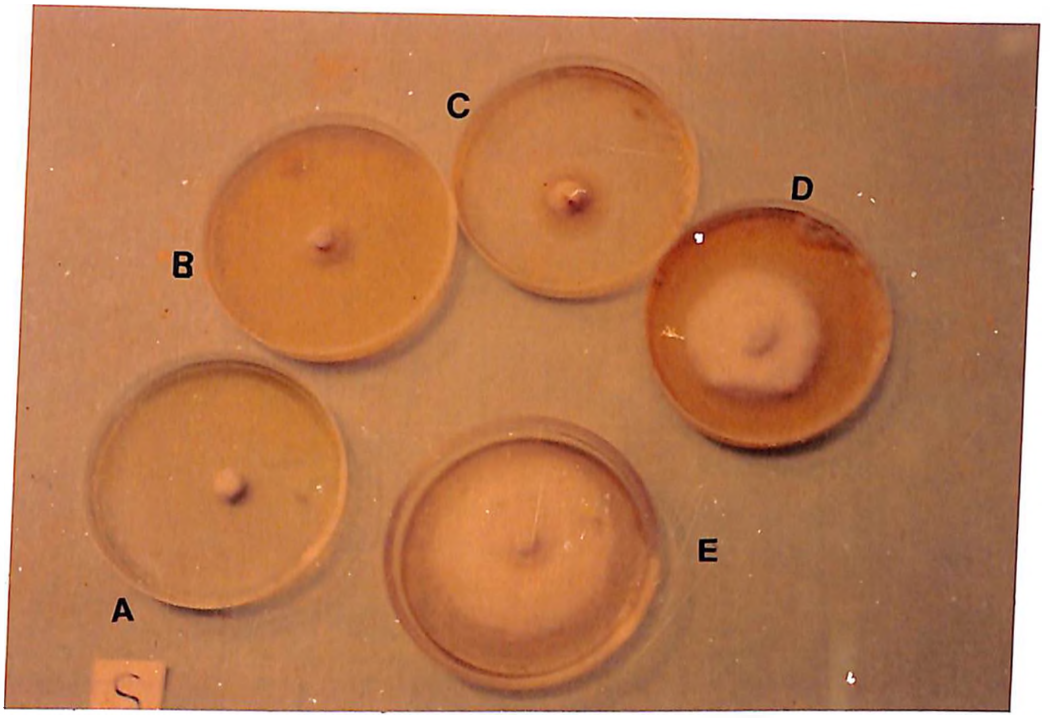

Plate 4(a). Cultures of *Colletotrichum coffeanum* in PDA amended with *Solanum nigrum* stem extracts from a four months old plants at four different concentration levels in (g/ml).

A = 0.2

B = 0.1

C = 0.05

D = 0.025

E = 0 (Control).

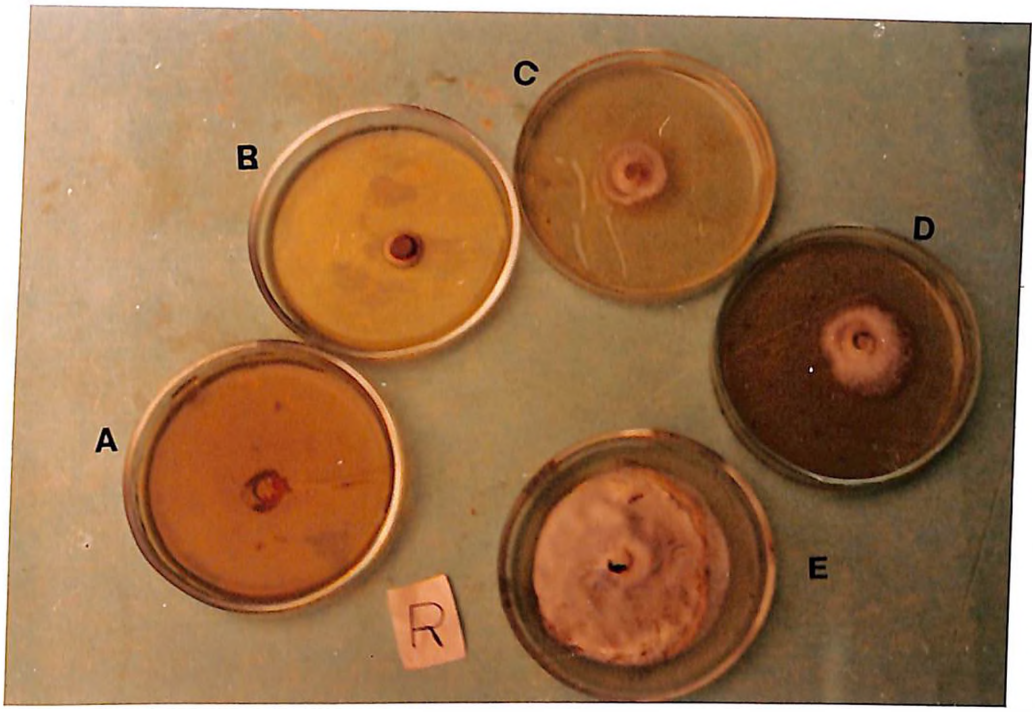

Plate 4(b). Cultures of *Colletotrichum musae* in PDA amended with *Solanum nigrum* root extracts at four different concentration levels in (g/ml).

A = 0.2

B = 0.1

C = 0.05

D = 0.025

E = 0 (control).

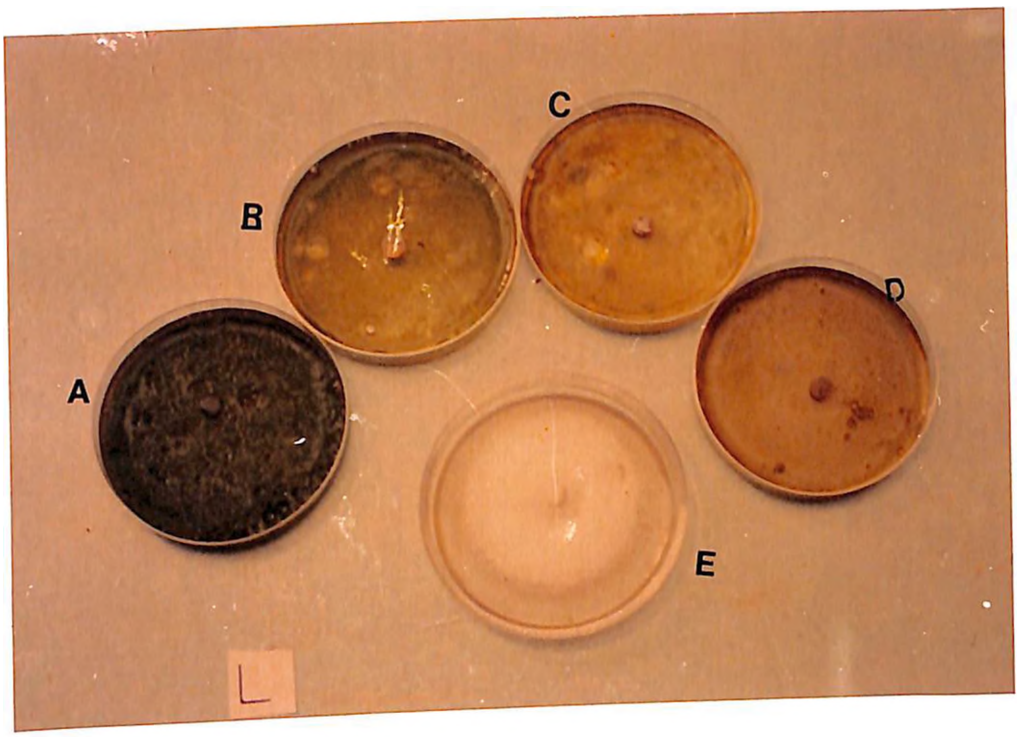

Plate 5(a). Seven day old *Colletotrichum coffeanum* in PDA amended with *Solanum nigrum* leaf extract from four months old plants at different concentration levels in (g/ml).

A = 0.2

B = 0.1

C = 0.05

D = 0.025

E = 0 (control).

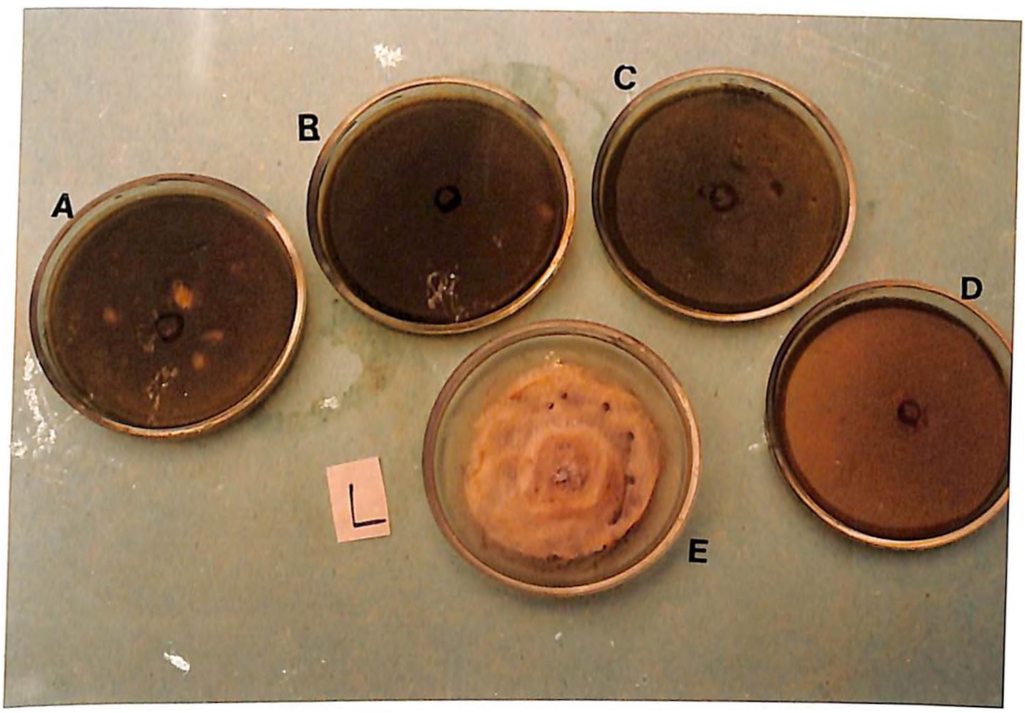

Plate 5(b). Ten day old *Colletotrichum musae* in PDA amended with *Solanum nigrum* leaf extract from a four months old plant at different concentrations in (g/ml).

A = 0.2

B = 0.1

C = 0.05

D = 0.025

E = 0 (control).

Table 8a. Effect of extract concentration on mycelial growth inhibition of the four species of *Colletotrichum*. Given are F and P values obtained from analysis (Anova) of the six different concentration levels used in the study.

A : *C. coffeanum* B : *C. gloeosporioides*
C : *C. musae* D : *C. lindemuthianum*

Stem[1] : Young part of stem
Stem[2] : Older part of stem

Age	Extract	Species	F	p
1	Leaf			
		A	0.0	p>0.05
		B	0.0	p>0.05
		C	0.0	p>0.05
		D	0.0	p>0.05
	Stem			
		A	472.4	p<0.05
		B	1251.24	p<0.05
		C	2320.12	p<0.05
		D	382.79	p<0.05
	Root			
		A	1136.51	p<0.05
		B	561.786	p<0.05
		C	509.75	p<0.05
		D	512.66	p<0.05
2	Leaf			
		A	0.0	p>0.05
		B	0.0	p>0.05
		C	0.0	p>0.05
		D	0.0	p>0.05
	Stem[1]			
		A	1668.47	p<0.05
		B	1331.4	p<0.05
		C	2001.2	p<0.05
		D	450.8	p<0.05
	Stem[2]			
		A	559.6	p<0.05
		B	2266.4	p<0.05
		C	1453.7	p<0.05
		D	382.8	p<0.05
	Root			
		A	558.7	p<0.05
		B	328.7	p<0.05
		C	446.8	p<0.05
		D	691.7	p<0.05
3	Leaf			
		A	0.0	p>0.05
		B	0.0	p>0.05
		C	0.0	p>0.05
		D	0.0	p>0.05

Table 8a continued.

Stem[1]			
	A	1481.3	p<0.05
	B	734.1	p<0.05
	C	890.0	p<0.05
	D	558.2	p<0.05
Stem[2]			
	A	1902.4	p<0.05
	B	824.7	p<0.05
	C	659.4	p<0.05
	D	1036.5	p<0.05
Root			
	A	1518.0	p<0.05
	B	479.5	p<0.05
	C	574.9	p<0.05
	D	1223.2	p<0.05
4			
Leaf	A	0.0	p>0.05
	B	0.0	p>0.05
	C	0.0	p>0.05
	D	0.0	p>0.05
Stem[1]			
	A	631.9	p<0.05
	B	197.6	p<0.05
	C	1349.7	p<0.05
	D	831.4	p<0.05
Stem[2]			
	A	670.1	p<0.05
	B	1591.0	p<0.05
	C	610.8	p<0.05
	D	431.9	p<0.05
Root			
	A	1471.0	p<0.05
	B	1008.0	p<0.05
	C	468.3	p<0.05
	D	703.2	p<0.05

Table 8b. Extract from fruits at different stages of development.

Extract	Species	F	p
Immature fruits	A	3458.1	p<0.05
	B	5219.4	p<0.05
	C	4589.4	p<0.05
	D	4055.7	p<0.05
Mature fruits	A	480.9	p<0.05
	B	679.7	p<0.05
	C	712.2	p<0.05
	D	1878.5	p<0.05
Ripe fruits	A	814.3	p<0.05
	B	690.6	p<0.05
	C	324.7	p<0.05
	D	1019.1	p<0.05

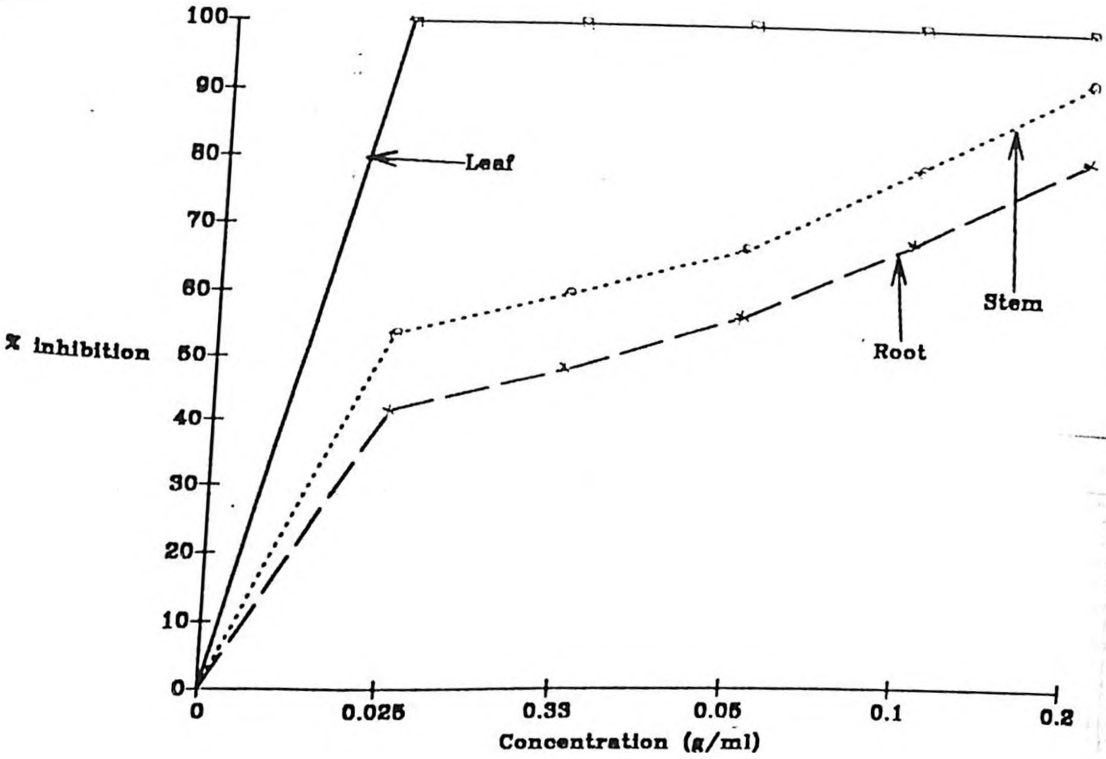

Fig. 2a Effects of concentration on mycelial growth inhibition of *Colletotrichum coffeanum*, by extracts from different parts of one month old *Solanum nigrum* plants.

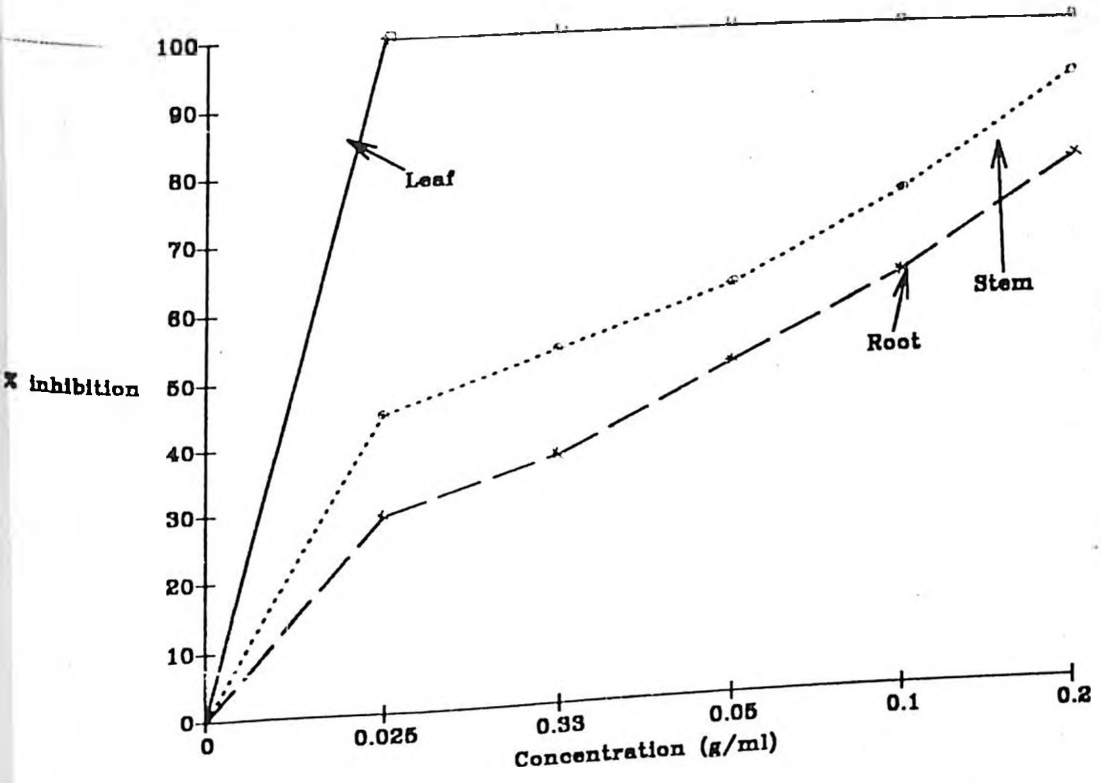

Fig. 2b Effects of concentration on mycelial growth inhibition of *Colletotrichum gloeosporioides*, by extracts from different parts of one month old *Solanum nigrum* plants.

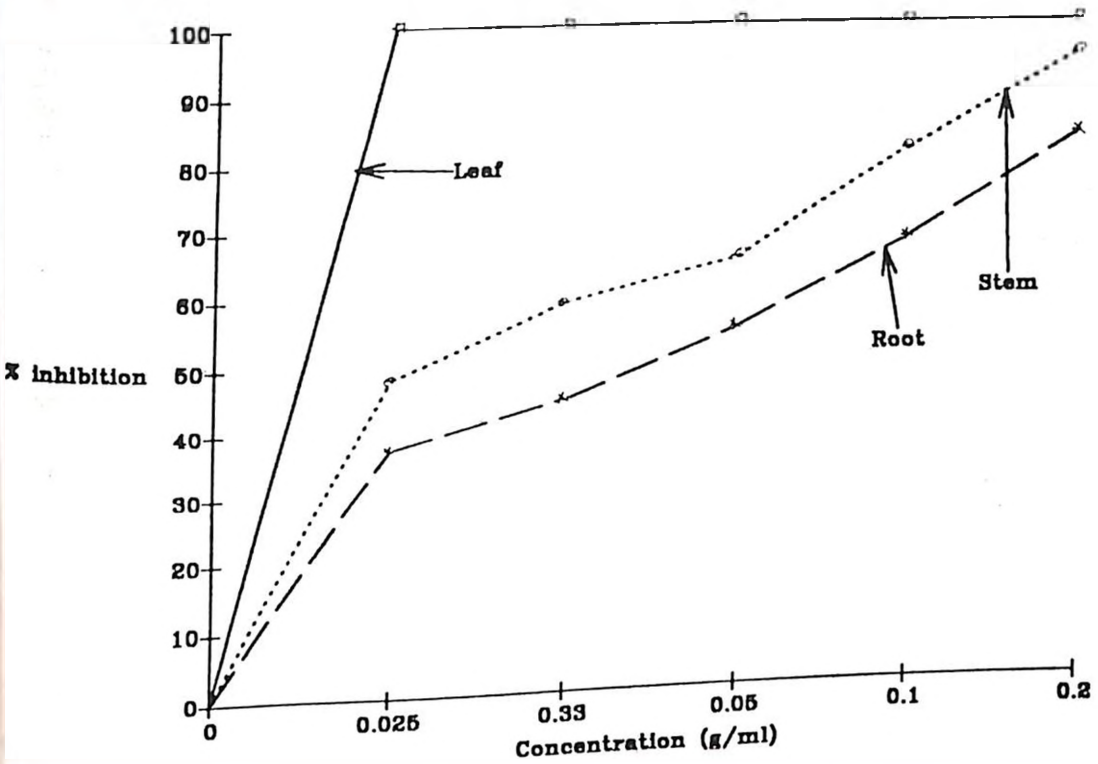

Fig. 2c Effects of concentration on mycelial growth inhibition of *Colletotrichum musae*, by extracts from different parts of one month old *Solanum nigrum* plants.

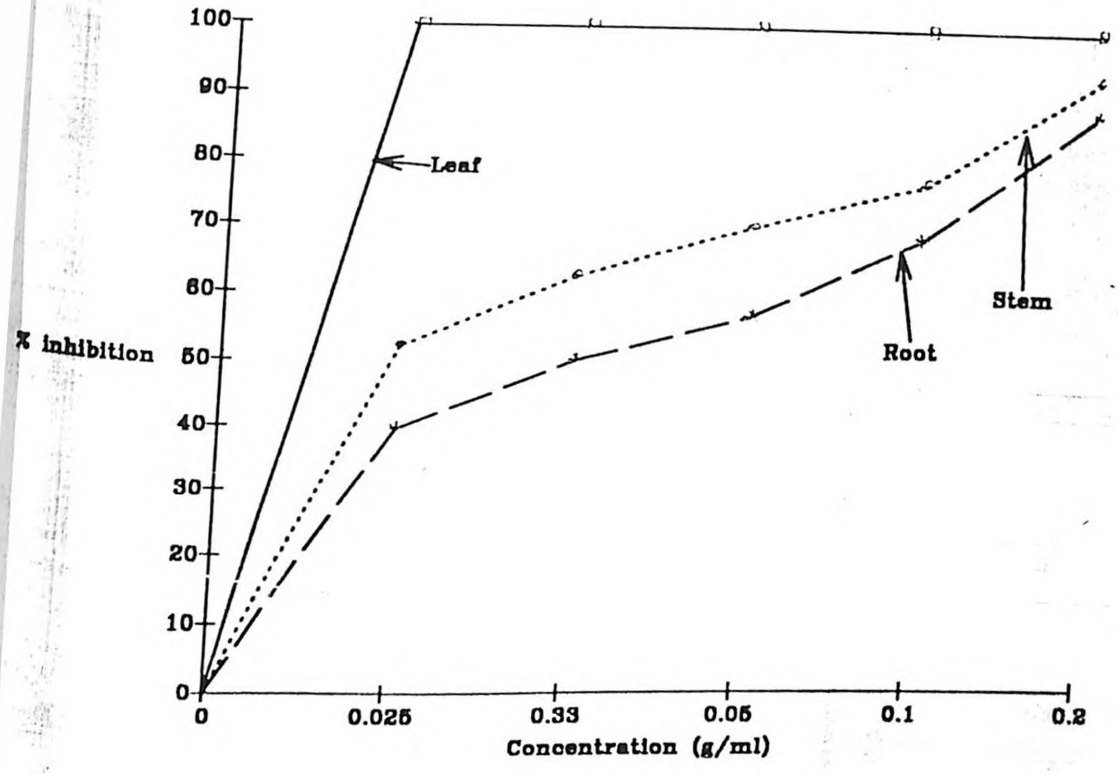

Fig. 2d Effects of concentration on mycelial growth inhibition of *Colletotrichum lindemuthianum*, by extracts from different parts of one month old *Solanum nigrum* plants.

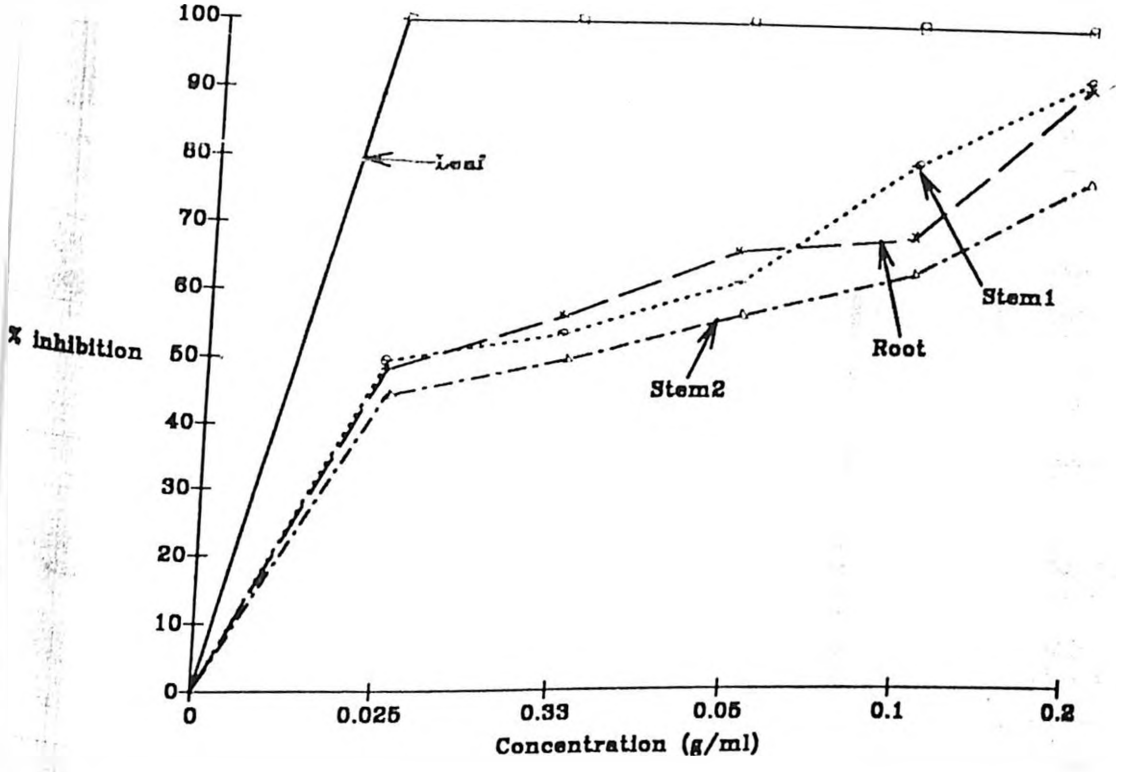

Fig. 3a Effects of concentration on mycelial growth inhibition of *Colletotrichum coffeanum*, by extracts from different parts of two month old *Solanum nigrum* plants.

Stem1 - Young part of stem
Stem2 - Older part of stem

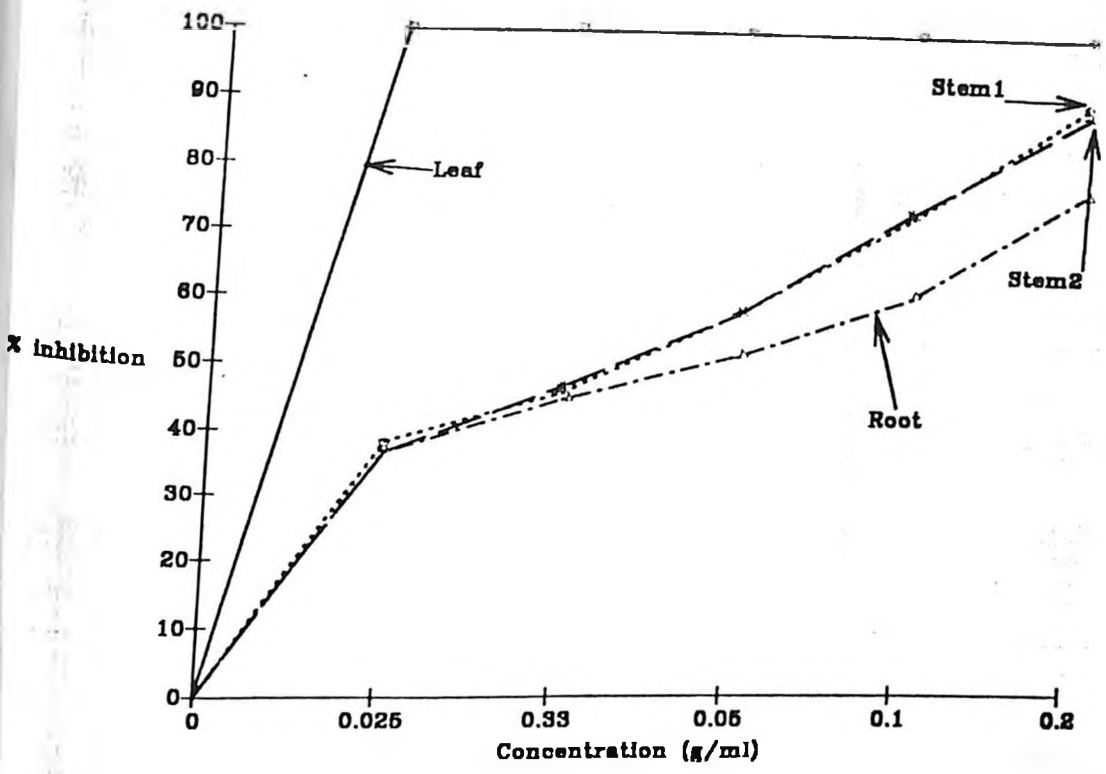

Fig. 3b Effects of concentration on mycelial growth inhibition of *Colletotrichum gloeosporioides*, by extracts from different parts of two month old *Solanum nigrum* plants.

Stem1 - Young part of stem
Stem2 - Older part of stem

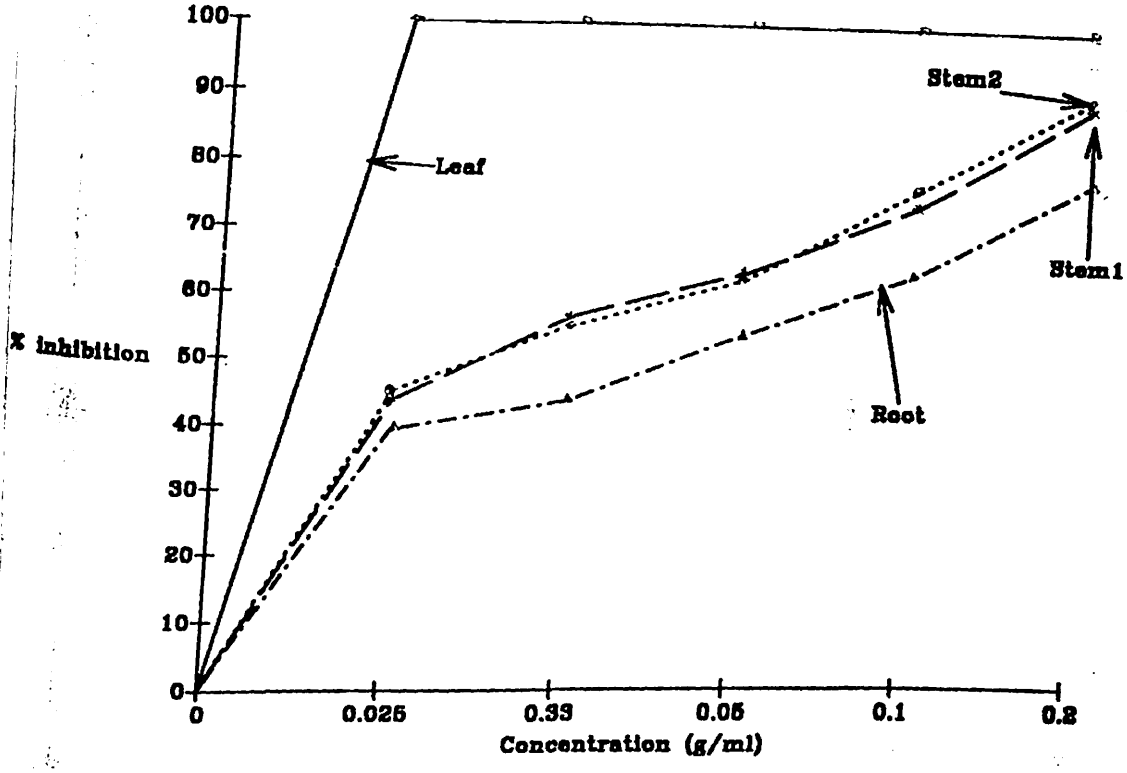

Fig. 3c Effects of concentration on mycelial growth inhibition of *Colletotrichum musae*, by extracts from different parts of two month old *Solanum nigrum* plants.

Stem1 - Young part of stem
Stem2 - Older part of stem

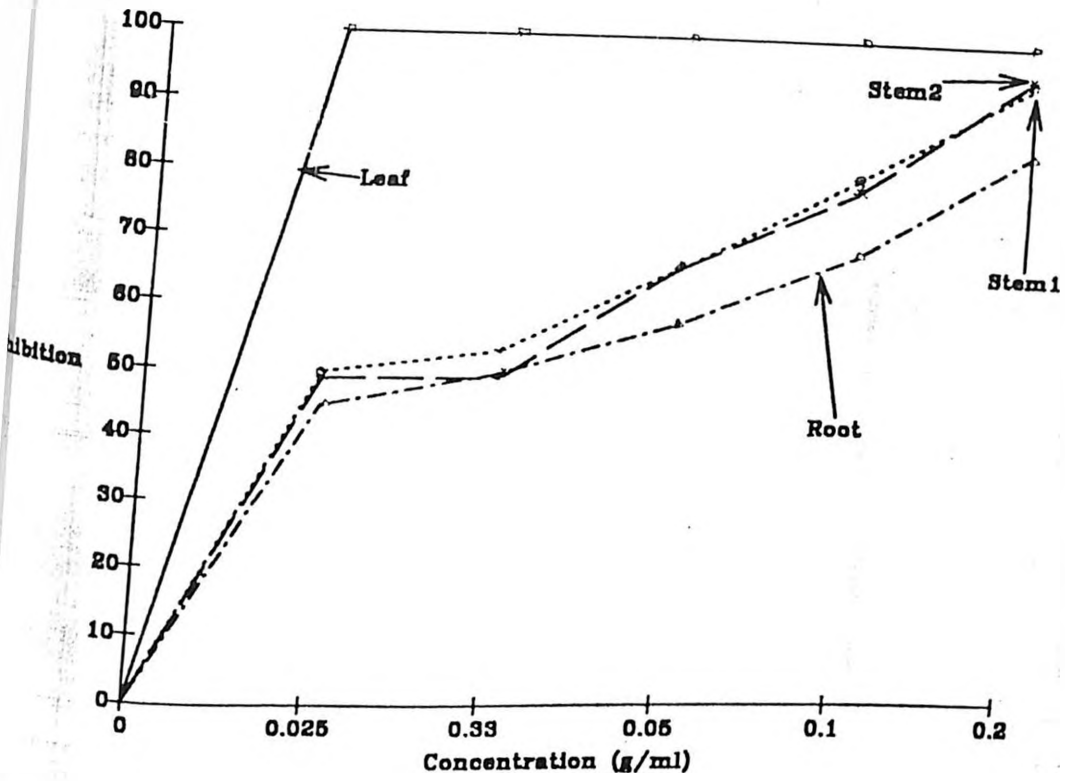

Fig. 3d Effects of concentration on mycelial growth inhibition of *Colletotrichum lindemuthianum*, by extracts from different parts of two month old *Solanum nigrum* plants.

Stem1 - Young part of stem
Stem2 - Older part of stem

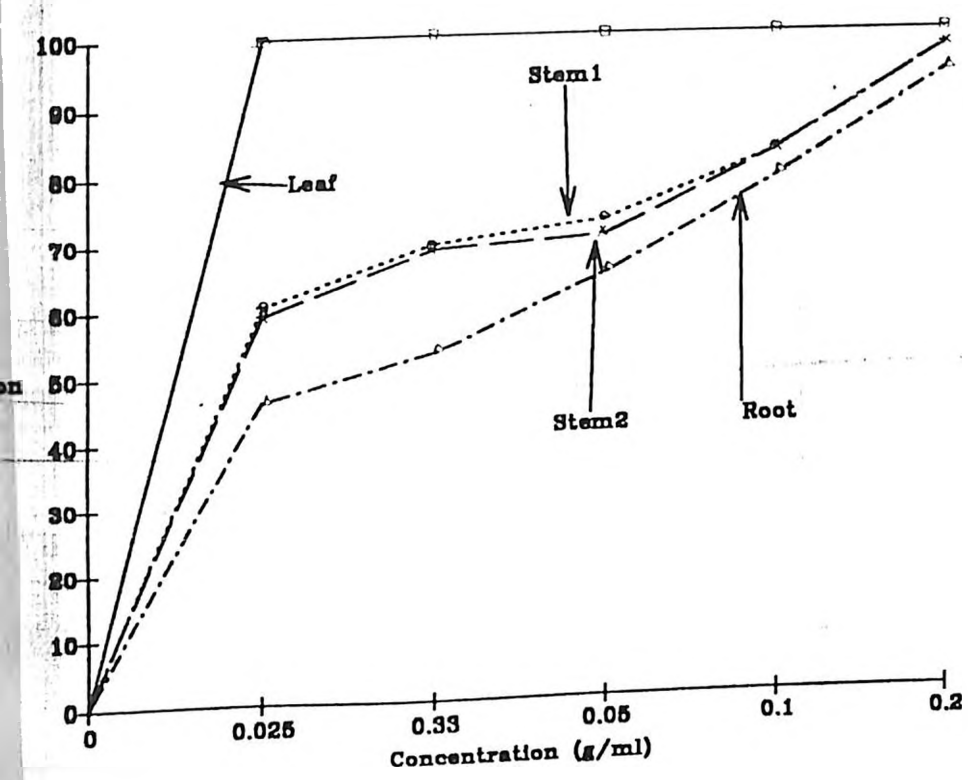

Fig. 4a Effects of concentration on mycelial growth inhibition of *Colletotrichum coffeanum*, by extracts from different parts of three month old *Solanum nigrum* plants.

Stem1 - Young part of stem
Stem2 - Older part of stem

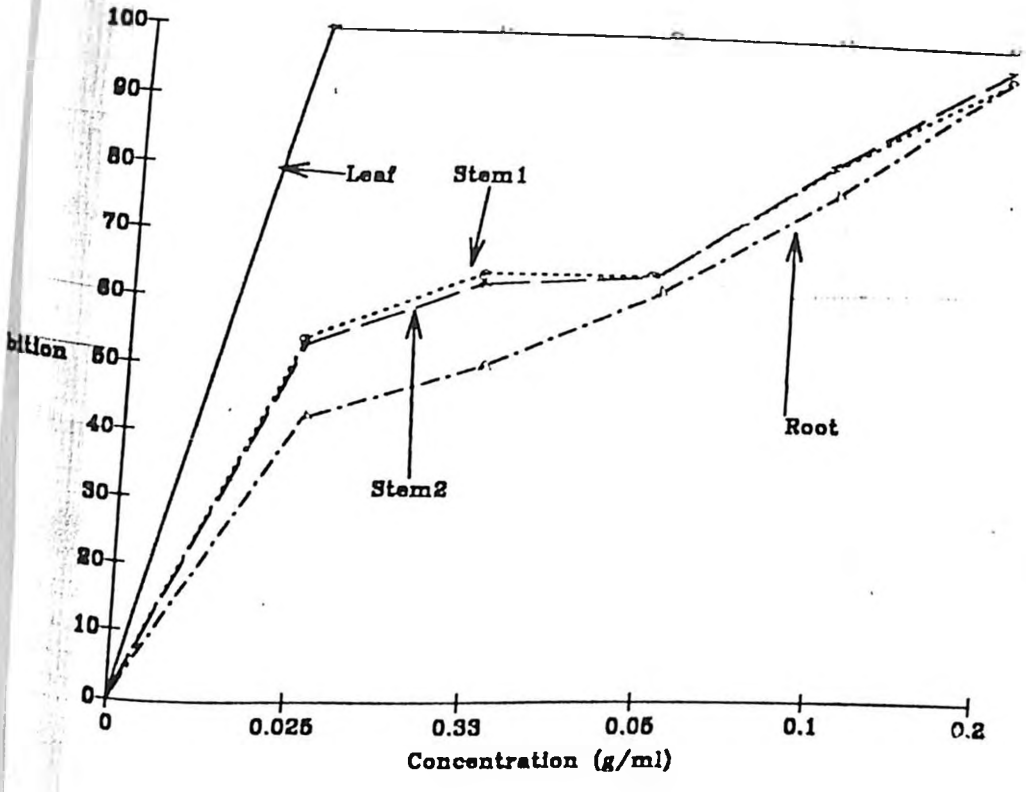

Fig. 4b Effects of concentration on mycelial growth inhibition of *Colletotrichum gloeosporioides*, by extracts from different parts of three month old *Solanum nigrum* plants.

Stem1 - Young part of stem
Stem2 - Older part of stem

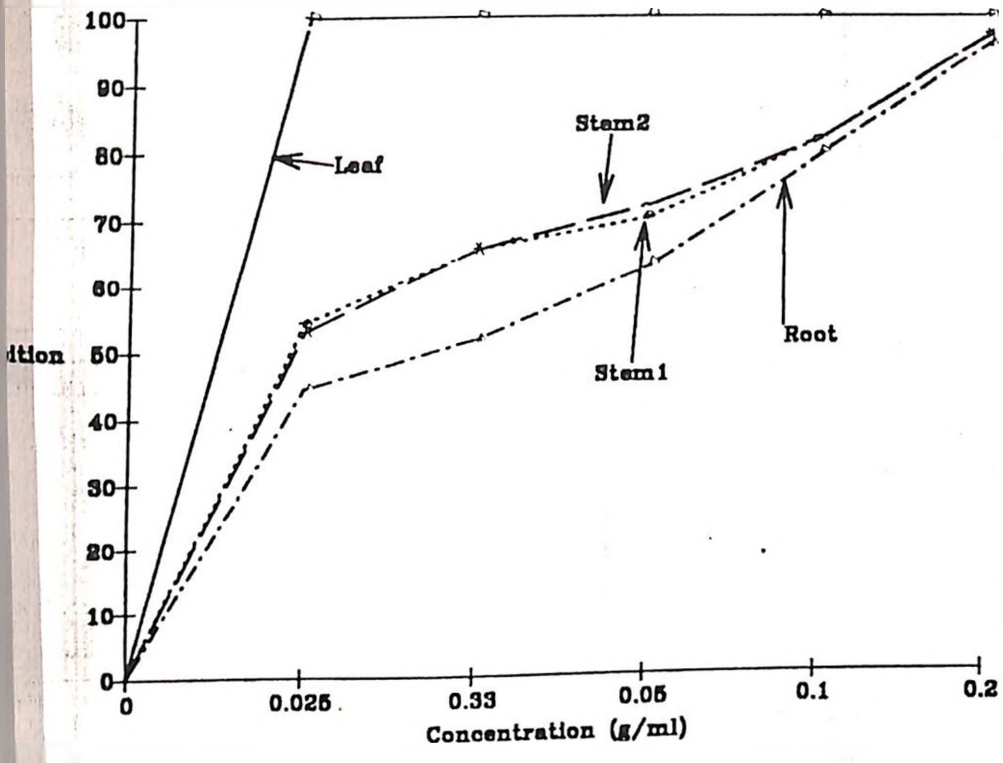

Fig. 4c Effects of concentration on mycelial growth inhibition of *Colletotrichum musae*, by extracts from different parts of three month old *Solanum nigrum* plants.

Stem1 - Young part of stem
Stem2 - Older part of stem

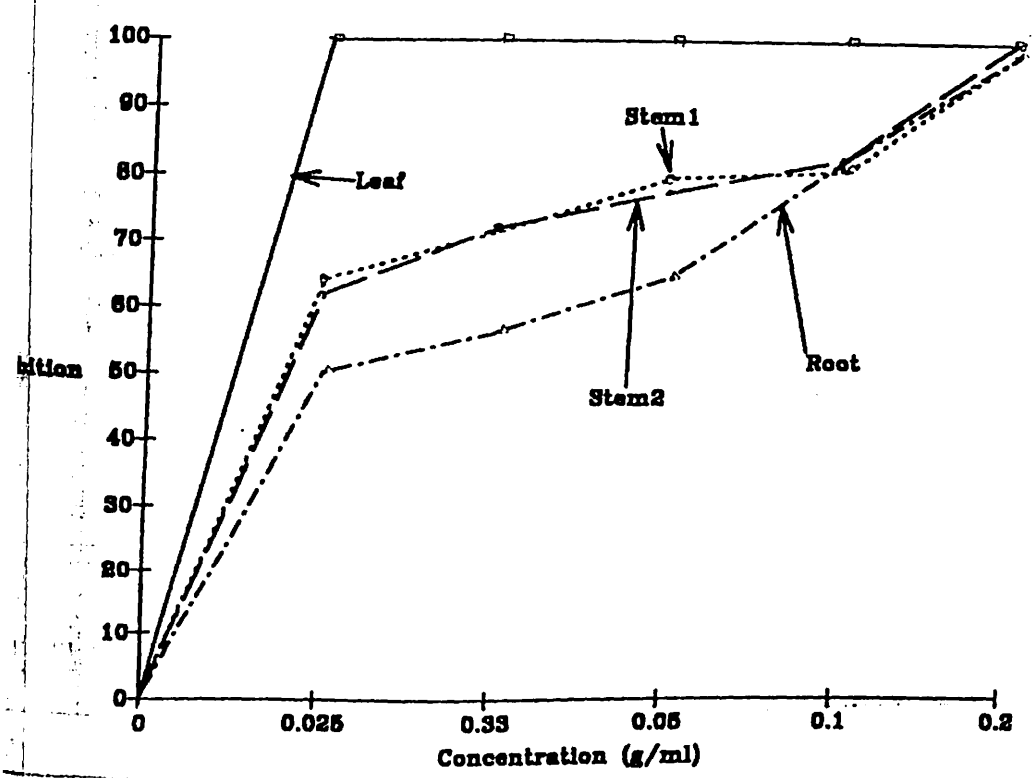

Fig. 4d Effects of concentration on mycelial growth inhibition of *Colletotrichum lindemuthianum*, by extracts from different parts of three month old *Solanum nigrum* plants.

Stem1 - Young part of stem
Stem2 - Older part of stem

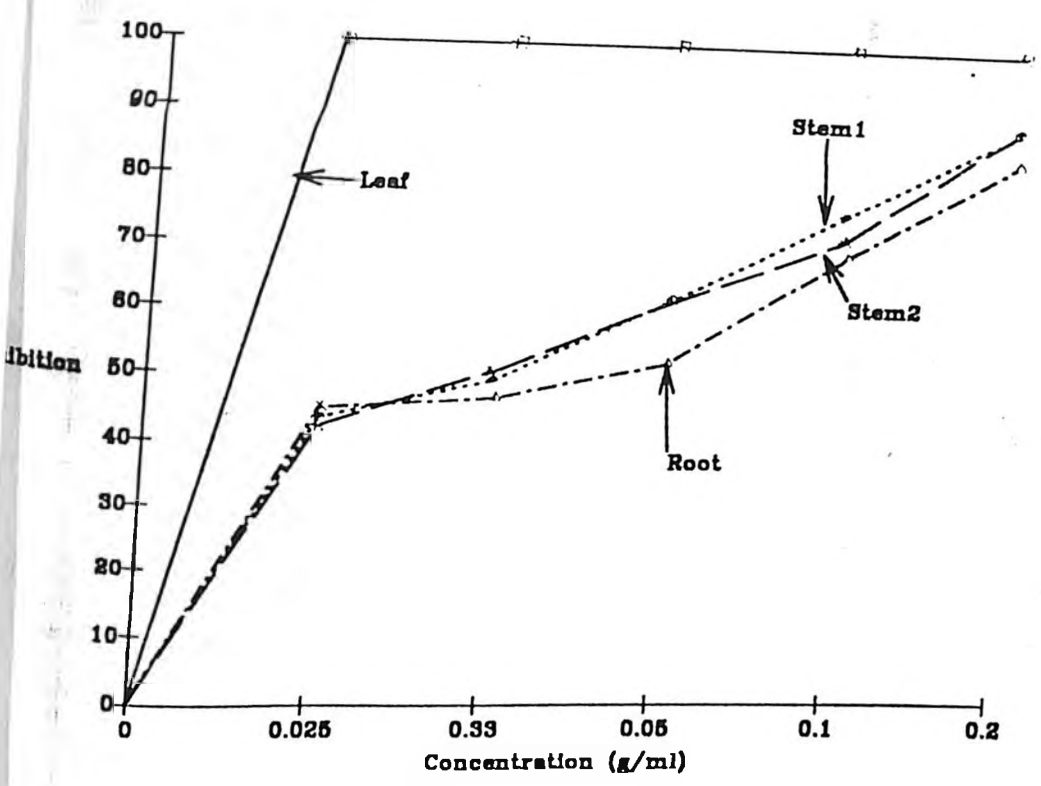

Fig. 5a Effects of concentration on mycelial growth inhibition of *Colletotrichum coffeanum*, by extracts from different parts of four month old *Solanum nigrum* plants.

Stem1 - Young part of stem
Stem2 - Older part of stem

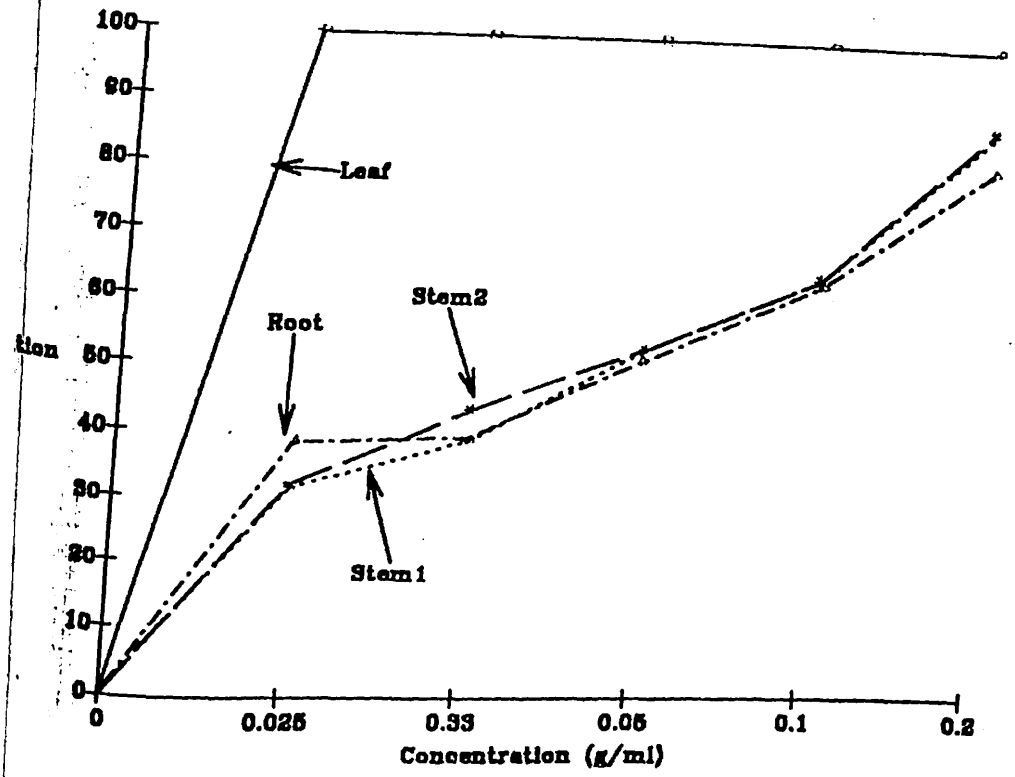

Fig. 5b Effects of concentration on mycelial growth inhibition of *Colletotrichum gloeosporioides*, by extracts from different parts of four month old *Solanum nigrum* plants.

Stem1 - Young part of stem
Stem2 - Older part of stem

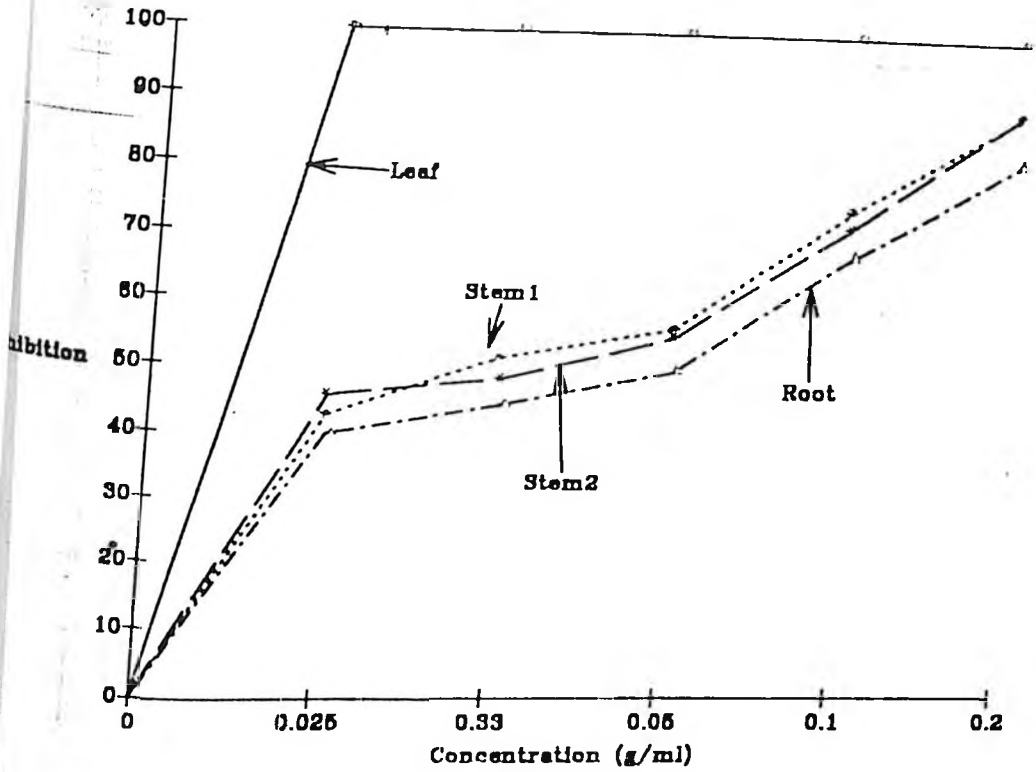

Fig. 5c Effects of concentration on mycelial growth inhibition of *Colletotrichum musae*, by extracts from different parts of four month old *Solanum nigrum* plants.

Stem1 - Young part of stem
Stem2 - Older part of stem

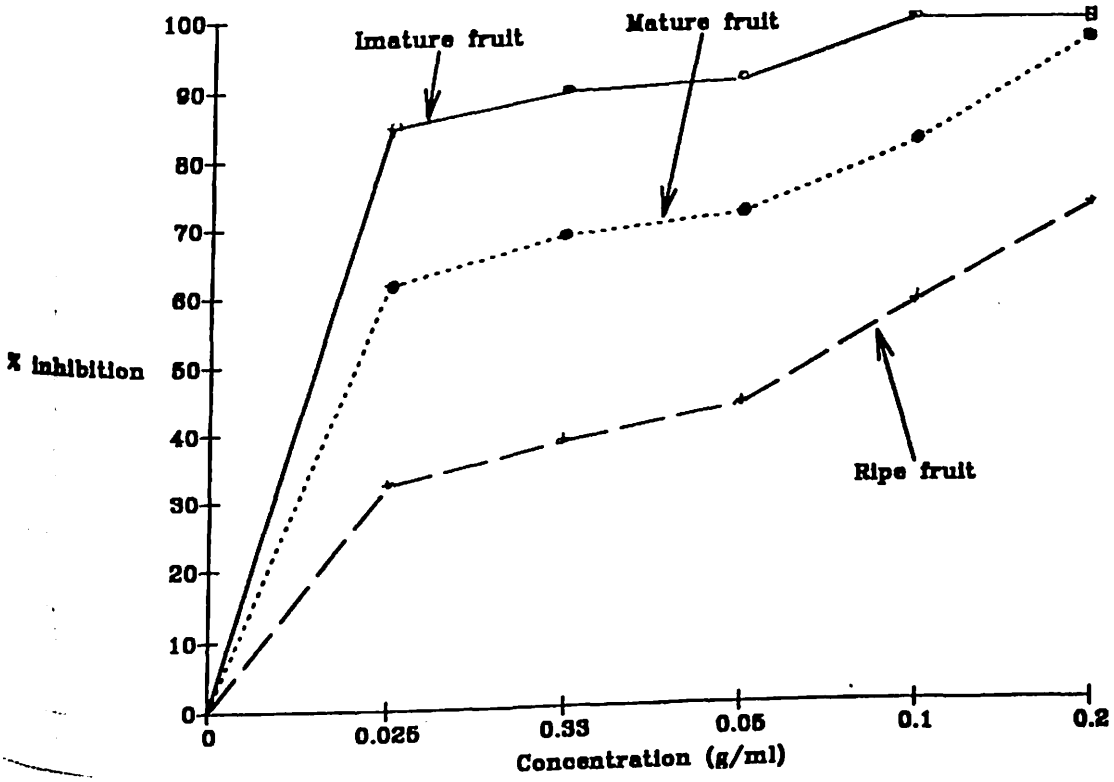

Fig. 6a Effects of concentration on mycelial growth inhibition of *Colletotrichum coffeanum*, by extracts from fruits at different stages of development.

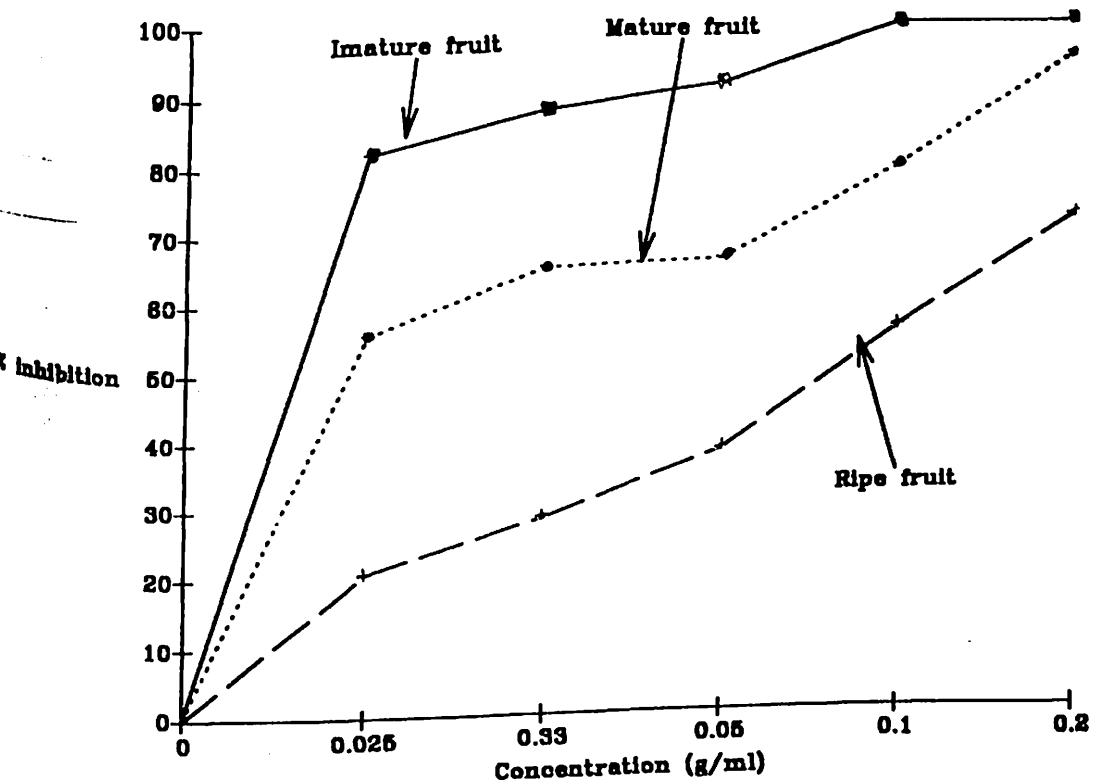

Fig. 6b Effects of concentration on mycelial growth inhibition of *Colletotrichum gloeosporioides*, by extracts from fruits at different stages of development.

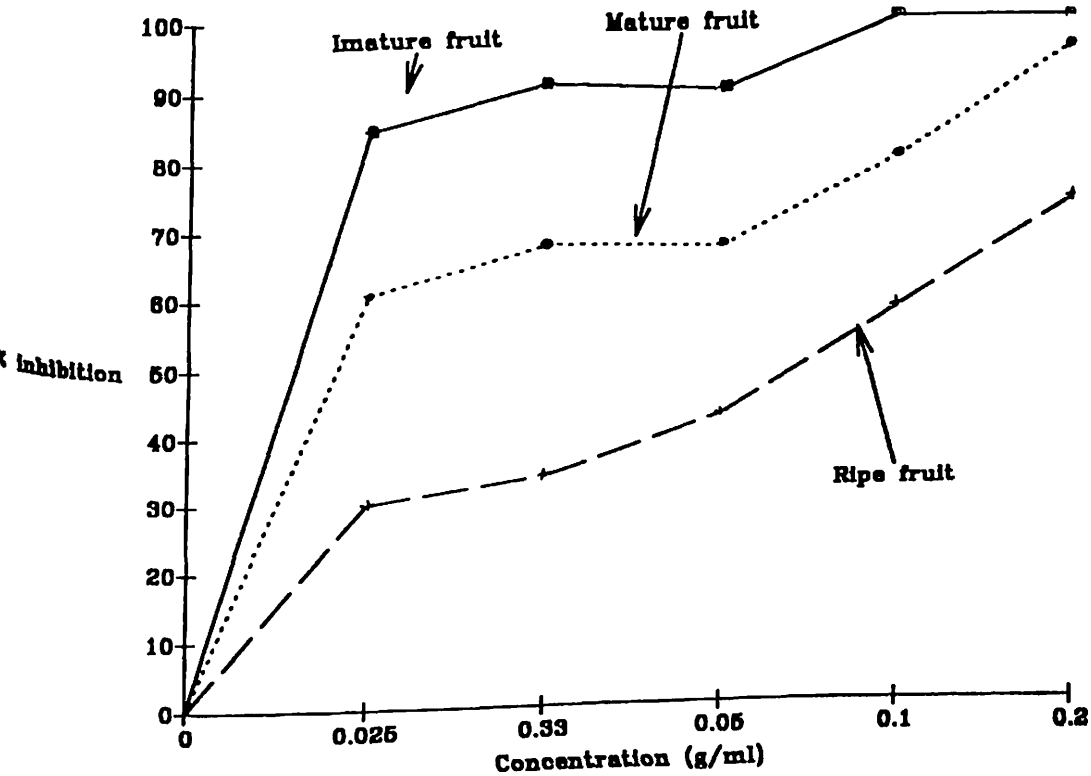

Fig. 6c Effects of concentration on mycelial growth inhibition of *Colletotrichum musae*, by extracts from fruits at different stages of development.

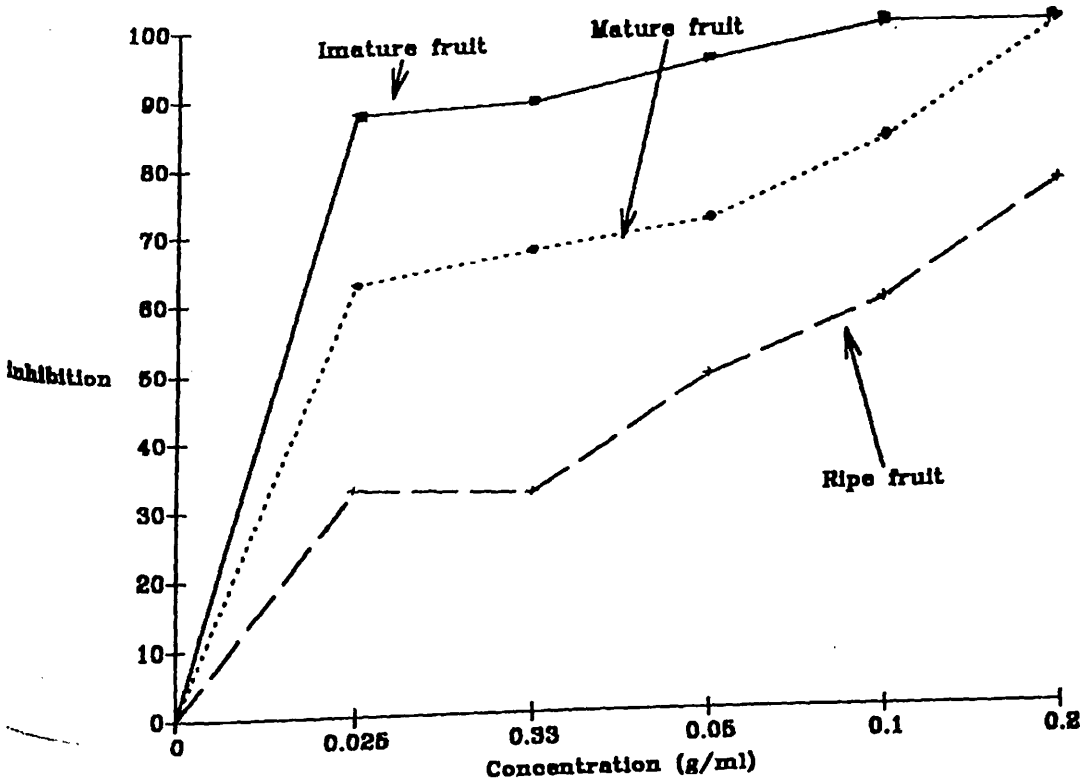

Fig. 6d Effects of concentration on mycelial growth inhibition of *Colletotrichum lindemuthianum*, by extracts from fruits at different stages of development.

Plate 6. *Solanum nigrum* leaves from a four months old plant at different stages of development.

I : young

II : middle

III : old.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSIONS

5.1 Discussion

Solanum nigrum leaf extracts completely inhibited the radial mycelial growth and sporulation of *Colletotrichum coffeanum, C. lindemuthianum, C. musae* and *C. gloeosporioides*. Extracts from stems, roots and fruits had varying degrees of growth inhibition amongst the different *Colletotrichum* species.

Of the extracts evaluated 27.8% of the cases showed that the differences in response between the four species were significant, and 72.2% of the cases showed that the differences in response by the different species were not significant. Whereas the significance of the 27.8% of the cases could be listed on their own merit, they must be treated with caution. They constitute an exception rather than the trend, and the apt conclusion would be that they were a divergent minority. As such the conlusion still holds that there was no significant differences in response to extracts by the different species in general.

The inhibitive property was not adversely affected after media amended with the leaf extracts were kept at 15 ° C, 20° C, 25° C, 30°C and 40° C for eight hours and then inoculated with the fungi. Extracts refrigerated at 4° C for one month also remained inhibitive. Extracts stored at room temperature for five days did not loose their inhibitive property but became contaminated by bacteria. Therefore *Solanum nigrum* extracts can be stored by refrigeration.

Radial mycelial growth of the *Colletotrichum* spp. on media amended with autoclaved extracts was as good as those on control plates. Thus autoclaving *Solanum nigrum* extracts completely destroyed the inhibitive property. This agrees with Muenscher (1951) who noted that there was a poisonous principle in the leaves and green fruits of *S. nigrum*, which was destroyed by boiling.

It is therefore evident that this principal is heat labile. This principle is probably the one responsible for inhibiting fungal mycelial growth. From the experimental tests , it is evident that *S. nigrum* extracts contain a fraction that is toxic to fungi and not bacteria. This is because the biggest problem encountered was that of bacterial contaminants. However further testing of the extracts on bacteria is necessary to confirm this.

Extracts made from different parts of *S. nigrum* had varying degrees of growth inhibition and suppression of sporulation on the test fungi. Inhibition by leaf extracts was significantly different from stem and root extracts. There was a significant difference in mycelial growth inhibition by stem and root extracts from plants of the same age. This was true for ages 1, 2, and 4. Whereas the inhibitive property of extracts prepared from stems and roots respectively differed significantly at different ages, inhibitions by leaf extracts remained 100 %. At age 3 there was no significant difference in fungal inhibition by extracts made from different parts of the plant. This probably suggests that the active antifungal ingredient was uniformly distributed in all plant parts at this age. There was a significant difference in fungal inhibition by extracts from fruits at different stages of development. The green fruits had a higher percentage inhibition than the red ripe fruits. It is therefore possible that the active antifungal ingredient is more in the green fruits and decreases as the fruit matures and ripens.

Other workers; Chiappeta *et al* (1988), Kumar and Sachan (1979), Kumar and Nene (1968) reported varying inhibitory effects on bacteria or fungal pathogens by extracts prepared from different plant organs. This probably suggests that different plant organs have different concentration of inhibitors. In this study it was evident that for the preparation of extracts with the best inhibitory effects, leaves from any age can be used, stems and roots from three months old plant and fruits at their youngest stage of development.

The finding that extracts made from the same part of the plant (leaves excluded) at different ages, differ significantly, probably suggests that the concentration of active antifungal ingredients

changes during the developmental stages of these parts of the plant.

For inhibitions by stem, root, mature green fruits and ripe fruit extracts, higher concentrations were more effective than the lower ones. With increase in concentration of the extracts radial mycelial growth and sporulation were inhibited to a greater extent. This is in agreement with the findings by earlier workers; Kuntz and Walker (1947) who found that inhibition of viruses by spinach (*Spinacia oleraceae*) extracts decrease with increased dilution of the extract. Kiragu (1983) working on the effect of different benomyl concentrations on *C. coffeanum* , found a decrease in the diameters of fungal colonies with an increase in benomyl concentrations. Johnson (1980) found that higher concentrations of guar (*Cymopsis tetragonoloba*) root extracts inhibited the growth of fungal pathogens to a greater extent. Tandon and Singh (1968b) in their study on the control of mango anthracnose with fungicides, also found that higher concentrations were more effective than the lower ones for all the fungicides tested. Rielly and Lamoureux (1981) in their study on the effect of the fungicide iprodione on the mycelium of *Sclerotinia sclerotiorum* found that the fungal colony diameters decreased with an increase on the concentration and that percentage inhibition increased with an increase on the concentration of the fungicide. This finding probably implies that the extracts were fungitoxic such that at high concentrations they were more toxic to the fungi than at low concentrations.

The presence of agar in a test medium has been shown to reduce the inhibitory effects of certain antibiotics and other antimicrobial substances. Ho and Ko, (1980); Hanus *et al*, (1967) found that solidifying agents contain sufficient nutrients to support complete or partial germination of fungi. However in this study, complete inhibition by some of the extracts was observed. Therefore, the mycelial growth, that was present in some cases cannot be attributed to the nutrients present in the solidifying agent (Agar) .

In this study, cold sterile distilled water extracts were used. Extracts made with other solvents could probably have differed in their inhibitive properties. McGray and McDonough

(1954) found that water extracts of *Catalpa speciosa* lost their antibiotic property rapidly but extracts of *C. speciosa* in alcohol retained their antibiotic capacities indefinitely. In this study, comparision of different solvents was not done, since the major objective of the work was to find out whether the plant *(Solanum nigrum)* contains any fungal inhibitors regardless of the solvent used in the making of the extracts. This objective was realised by using the cheapest solvent, it was therefore not necessary to go into comparisions of solvents.

Inspite of the *in vitro* effectiveness of extracts from *S. nigrum*, it is not known if the antifungal substances from *S. nigrum* would effectively reduce the severity or incidence of plant diseases in the field caused by the four species of *Colletotrichum* that were sensitive in the study. Therefore,further experiments on their ability to control fungal diseases *in vivo* are required before definitive statements on their agricultural usefulness can be made. This is because it has been reported that some fungicides can be effective *in vitro* but not in the field (Vine *et al* 1973b). Sheodhan *et al* (1979) also reported that extracts of *Nicotiana tabacum* gave an effective control of hill bunt of wheat in pot experiments but failed to control the disease under field conditions.

It has also been reported that some crude extracts from plants show positive and very encouraging results but when the active compound is finaly isolated, it proves to be very unstable (Green *et al*, 1977). Therefore, further studies are needed to isolate and characterise the antifungal substances from *S. nigrum.* The antifungal substance may then be tested both *in vitro* and *in vivo* on the species of *Colletotrichum* and other fungal pathogens that are of economic importance. This might ultimately help in evolving a new effective fungicide for the control of *C. coffeanum, C. lindemuthianum, C. musae, C. gloeosporioides* and possibly other phytopathogenic, human and animal fungal pathogens.

It was observed that when mycelial innoculum plugs were transferred into extract free media, some plugs resumed growth and others did not. This was dependent on the concetrations of the extracts in which the plugs had initialy been incubated. Mycelial plugs transferred from plates

treated with higher extract concentrations completely lost viability whereas those transfered from plates that had been treated with lower concentrations were still viable but the growth was retarded. Hence it may be possible that the extract were fungicidal at high concentrations and fungistatic at low concentrations. Therefore as a fungicide *S. nigrum* extract could possibly be classified as a protectant. This group of fungicides protect the plant by killing or inhibiting the development of spores or mycelium at the site of infection (Evans, 1968). However *in vivo* experiments are necessary to confirm this finding. From this study it's clear that most of the active ingredients in *S. nigrum* could be found in the leaves and green fruits. This is due to the fact that extracts from these parts had the highest inhibitory effects. From the experimental tests it also appeared that the antifungal substance was more in the young fruits and may have been metabolised as the fruits matured and ripened. The inhibition by the ripe fruit extracts was very low. This finding agrees with that of Muenscher (1951), who noted that there were poisonous alkaloids in the fruit of *S. nigrum* which disappeared as the fruit ripens. Disappearance of compounds in ripening seeds in a number of plants has already been mentioned. Floss *et al* (1974) noted that alkaloids from the fruits of *Nicotiana* spp disappeared with ripening. They also noted that the steroid alkaloids present in the fruits of *Solanum dulcamara* are metabolised almost completely as the fruit ripens.

During the plant growth stages, the fluctuations of the active ingredient content in the extracts of *S. nigrum* (excluding those from leaves) was significant. This agrees with findings by Hughes and Genest (1973) who noted that alkaloid content of many plants including some members of the family Solanaceae fluctuated markedly during the growing season. Hughes and Genest (1973) noted that there was appearance and disappearance of alkaloids in some plant organs during growth. He suggested that probably the alkaloids are translocated to another site, broken down in situ, exhaled or leached out. He also noted that there were diurnal changes which could be considerable in some cases . In *Lupinus albus* for example, alkaloid content increases during day and decreases at night. In this study, the effect of diurnal changes was not investigated as the plants were

harvested only during the day.

Floss *et al*, (1974) noted that external factors such as climatic conditions, photoperiod, geographical location and an influence of a large allay of added chemicals have some influence on alkaloid production in plants. These factors may have positive or negative effects on alkaloid formation.

Alkaloids that have been isolated from *S. nigrum* include solanine, solamargine, ß-solamargine , α- and ß - solanigrine and solanine (Schreiber 1968). Solanine has been extracted from several other *Solanum* spp (Mbaya and Muhammed, 1976; Watt and Breyer -brandwijk, 1962). Torgeson (1969) suggested that solanine in potato tubers could be important in restricting *Fusarium* infection. Hence the compound has antifungal properties.

5.2 Summary and Conclusion

It was evident from this study that *Solanum nigrum* plant extracts have inhibitory effects against the four species of *Colletotrichum*. The differences in response to the extract by the four species were not significant.

Extracts obtained from different plant organs had varying degrees of inhibition on the fungal species. Leaf and green fruit extracts had the highest inhibitory effects against all the four species, while ripe fruits had the lowest.

Extracts from three months old plants had higher inhibitory effects than the other ages. Dilution of the extracts had an influence on the inhibitive property. Higher concentrations of the extracts had better inhibitory effects than lower concentrations. This was however not true of the leaf extracts for the concentration levels used in this study.

From this study, there is sufficient grounds for the conclusion that *Solanum nigrum* could be a potential source of a fungicide against the four species of *Colletotrichum*.

If field tests are carried out and the extracts are found to inhibit the fungal species on their natural hosts, then the next step would be to look for possibilities of large scale extraction, refinement and storage, and *in vivo* tests with the pure compound. The raw material (*Solanum nigrum*) being naturally abundant, easy and cheap to cultivate, it would be a reliable source of a new natural fungicide for these crop diseases.

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Appendix 1.

Media preparation.

Potato Dextrose Agar.

potatoes	200 grams
Agar	20 grams
Dextrose	15 grams
Distilled Water	1 Litre

Water Agar.

Agar	20 grams
Tap Water	1 Litre

The potatoes were boiled till soft and was then filtered through cheese cloth. To the filtrate agar was added and heated to dissolve then dextrose was added. The volume was adjusted with distilled water to one litre. This was then sterilised in the autoclave for 20 minutes at 121° C. For water agar, the agar was dissolved in distilled water and autoclaved for 20 minutes at 121° C.