STUDIES ON THE SCREENING OF SOME GHANAIAN PLANTS FOR FUNGITOXIC ACTIVITY AGAINST FIVE FUNGAL PATHOGENS.

A THESIS PRESENTED

BY

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ABSTRACT

The effect of the aqueous and methanol extracts of 12 Ghanaian plants belonging to 10 families on some aspects of the physiology of *Sclerotium rolfsii, Helminthosporium sp., Aspergillus niger, A. flavus* and *Fusarium oxysporium* were investigated in vitro in either liquid broth or agar medium amended with varying dilutions (undiluted, 1:1 - 1:5 v/v) of the extracts.

Comparatively higher fungistasis against *Sclerotium rolfsii* and *Helminthosporium sp.* were found in both aqueous and methanol extracts of the dry leaves of *Cassia alata, Abrus precatorius~i.* ajid *Desmodium triflorum.* The remaining plants (*Azadirachta indica, Alternanthera pungens, Boerhavia diffusa, Catharanthus roseus, Clausena anisata, Sida acuta, Mitragyna inermis, Oxalis corniculata* and *Zanthoxylum xanthoxyloides* exerted minimal inhibitory effect on the test fungi. In most instances, inhibitory effect was gradually removed with dilution of the extract and the minimal effects was even severer on *Helminthosporium sp* than *S. rolfsii.* Growth of test fungi in liquid medium did not differ significantly (P s 0.05) in liquid and on agar.

The test plants significantly depressed sclerotia production in vitro especially at higher concentration (1:1 and 1:2 v/v dilution) and the inhibitory effect was gradually removed with increasing dilution of the

extracts. In some instances, the methanol extracts were more effective than the aqueous extracts. It required a minimum contact period of 3 0 minutes with the extract of *C. alata* to permantly impair vegetative growth of *S. rolfsii.* The longer the immersion period in the extracts of *C. alata* the severer the depression in the dry matter accummulation by the fungus. Both vegetative growth and sclerotia production by *S. rolfsii* was completely prevented when the mycelium was buried in methanol extract of *C. alata* for 48 h prior to transfer into extract-free medium. Vegetative growth of *Helminthosporium sp.* similarly treated was depressed by 20.49 percent.

Further studies with the extracts of *C. alata* and *A. precatorius* showed that they could also variably depress vegetative growth of *Aspergillus niger, A. flavus* and *Fusarium oxysporium.* Practical implication of these findings are discussed and sequel studies suggested.

DECLARATION

I hereby declare that, except for references to other people's work which have been duly cited, this work is the result of my own original research and that this thesis has neither in whole nor in part been presented for another degree elsewhere.

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D E D I C A T I O N

Dedicated to my beloved husband Kofi, for his love, patience, support and encouragement throughout this research work and to my sweet little son, Nana Kwame.

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INTRODUCTION AND LITERATURE REVIEW

Over the years, several interesting and significant reviews have appeared on bio-pesticides and biofungicides (Van Latum and Gerrits, 1991; Jacobson and Crosby, 1971). The number of literature references concerned with the subject exceed 5,000. Several thousand species of higher plants and an unknown number of fungi and lower plants have been screened. (Van Latum and Gerrits, 1991).

The major pest and pathogens taking a heavy toll on agricultural crops in the field and in storage are insects and fungi. The idea of controlling them by the use of chemical is not new. However, many of the original chemicals developed generally become injurious to the environment with non-specific activity (Cremlyn, 1978) . There is now greater awareness of the dangers of environmental pollution arising from widespread application of chemicals; pesticides and fungicides (Carson, 1963) and candidate chemicals have to pass increasingly stringent test on their toxicity, and residue formation before they can be marketed as pesticides and fungicides in many countries. This has provided impetus in research on new agrochemicals with the view of applying naturally derived compounds which are safer and more selective in their action and do not affect non-target organisms.

Most plants contain secondary metabolites with peculiar individual properties, and often having no known relation

to the metabolism or function of the plant as a whole. Such plant constituents may vary from one species to another (Githens, 1949). When such a substance exerts an influence on the structure or function of another organism, it is known as an "active principle" (Githens, 1949) . It is the presence of such principles which provide the therapeutic value of plants. Recently, Penso (1982) estimated that 20,000 plants are used for therapeutic application.

The need to evaluate the biological activities of extracts and phytochemical constituent is not only important for the development of new therapeutic agents but also the new chemicals isolated from the plants with some biological activity become a springboard for chemist exploring plants for lead compounds to manufacture synthetic analogues from these naturally occurring compounds.

The use of angiosperm plant extracts to control microorganisms have been reported by many workers (Mbela et. *al. 1992;* Oji et al 1992; Lemos et *al.* 1992; Oloke, 1992; Saskena and Tripathi 1986; Tomes et *al.* 1986 etc). The extracts tested contain volatile oils (Methela and Methela, 1984) and xanthones (Cardona et al. 1985), tannins, alkaloids, steroids lactones (Kirson et al. 1970, 1971; Sabir et *al.* 1987), flavonoids (Krishnappan and Scetharaman, 1992) and coumarins (Tsitsa-Tzardi et *al.* 1992 etc.) . Because of the variation in chemical

components, each plant differ in their fungistatic and antibacterial activities and leaf extracts have been found to be generally more repressive than extracts from flowers, stems and roots (Saksena and Tripathi, 1986) . Plants from many families have been tested including members of families Aspidiaceae, Polypodiaceae, Sinopteridaceae, Acanthaceae, Agavaceae, Amaranthaceae, Anacardiaceae, Amaryllidaceae, Apocynaceae, Bignoniaceae, Caesalpinaceae, Compositae, Euphorbiaceae, Papilionaceae, Malvaceae, Meliaceae, Nyctaginaceae, Oxalidaceae, Rutaceae, Urticaceae and Violaceae (Chhabra et al. 1987; Apertorgbor, 1991). Fungi tested include *Aspergillus fumigatus, A. niger, Rhizopus arrhizus, Mucor mucedo, Alternaria alternata* (Saskena and Tipathi 1986) , *Helminthosporium nodosum, Sclerotium rolfsii, Fusarium oxysporium, Rhizoctonia solani* and *Phytophthora palmivora* (Singh et *al.* 1987; Myles, 1986; Boateng 1986).

As part of an on-going research in this laboratory Apetorgbor (1990) tested nineteen Ghanaian plants in the families Compositae, Papilionaceae, Caesalpinaceae, Rutaceae, Meliaceae, Malvaceae, Amaranthaceae, Nyctaginaceae, Oxalidaceae, Euphorbiaceae, Asclepiadaceae and Apocynaceae for their fungistatic and antimicrobial activities. Vegetative growth of the test fungi *(Scopulariopsis brevicaulis, Aspergillus niger, A. flavus, Sclerotium rolfsii,Nigrospora sp.)* treated with aqueous and methanol extracts of the plants for varying period

were variably depressed by the extracts. The longer the period of immersion the greater the depression of the vegetative growth (Apetorgbor,1991). Comparative higher fungistatic effects were found in both aqueous and methanol extracts of dry leaves of *Cassia rotundifolia, Pergularia daemia, Alternanthera pungens, Voacanga africana, Launaea taraxacifolia, Tridax procumbens, Zanthoxylum xantholoides, Oxalis corniculata, Azadirachta indica, Desmodium triflorum, Euphorbia heterophylla and Crotalaria retusa* (in decreasing order) on solid agar medium than in broth (Apetorgbor,1991). It was expedient to follow up these findings.

In this sequel study, twelve plants belonging to ten families listed in Table IB were selected on the basis of their active principle components and therapeutic applications reported in the literature. The yield of the active principle may vary depending on the solvent used for extraction. For example, Rao and Ahamed (1992) found that *Dalbergia paniculata* leaves yielded triacontanol (0,01%) and S-sitosterol (0,013%) in light petrol extract,- acetone extraction gave (+) pinitol (0.018%), apigenin (0.00125%), luteolin (0.0015%) and 7-O-glucosylcaviunim (0.0035%). Methanol extraction yielded 4,7-di-O-glucosylapigenin (0.00325%). On methylation followed by acid hydrolysis the compound gave 5-O-methylapigenin (Rao and Ahamed,1992).

TABLE 1

PLANTS USED: THEIR ACTIVE PRINCIPLES AND MEDICINAL VALUES

Plant species	Therapeutic applications	Active Principles	References
1. *Abrus precatorius* (Papilionaceae)	Asthma, whooping cough, eye sores, iritis, conjunctivitis, ophthalmia.	Abrine Abric acid	Ayensu(1978) Abbiw (1990)
2. *Alternanthera pungens (repens)* (Amaranthaceae)	snake bite, enema, headache, abortifacient sore-throat, dysentery, rheumatism.		Dokosi(1969) Dal zi el (1936) Ampofo(1983)
3. *Azadirachta indica* (Meliaceae)	cough, cold, fever, gastric disorders, anthelminthic, antibacterial.	nimbolin, tannin, glucosides, nimbin, nimbidin, azadi rachti n.	Ayitey-Smith (1989) Oliver-Beaver (1986)
4. *Boerha via diffusa* (Nyctaginaceae)	fever, laxative, convulsion, asthma, poultice, guinea worm, expectorant yaws, diuretic.	hentri acontane si sterol, ursolic acid hypoxanthine-9-L ■arabinofuranoside boerhavic acid, punarnavine, tannins, phlobaphenes.	Dal zi el (1936) Ojewole and Adesina (1985) Mizra and Towari(1971) 01i ver(1959) Ayensu(1978)

Plant species	Therapeutic applications	Active Principles	References
5. *Cassia alata* (Caesalpinaceae)	ring worm, snake bite, scurvy, skin diseases, laxative.	chrysophanic acid, anthraquinones, 3-sitosterol rhein, kaempferol, amino acids, coelulatin, azulene.	Holland(1922) Githens(1949) Abbiw (1990) Ogunti *et al* (1991)
6. *Catharanthus roseus* (Apocynaceae)	anti leukaemia, carcinoma of breast, Hodgkin's diseases, Wilm's tumour.	leurocri stine, vinblastine, vincristine, serpentine, Ajmalicine, Vindicine.	Sofowora(1982) Irvine (1961)
7. *Clausena anisata* (Rutaceae)	anthelmintic analgesic, antiseptic, iritis, ophthalmia, trachoma, insecticide, piles, headache, nasal drop.	helletine, imperatorin, scoparone, atanisatine, clausanitine, mupami ne.	Irvine (1961) Odamtten *et a 1* (1988)
8. *Desmodium triflorum* (Papilionaceae)	dysentery, expectorant, febrifuge, anti spasmodic, wound dressing, diarrhoea.	tannins.	Holland (1922) Githens (1949) Datta and Benerjee(1979)

Plant species	Therapeutic appli cati ons	Active Principles	References
9. *Mitragyna inermis* (Rubiaceae)	analgesic, gastro-intestinal, pains, diuretic, febrifuge.	mitrinermine. mitrinecomine.	Irvine (1961) Abbiw (1990)
10. *Oxalis corniculata* (Oxalidaceae)	astringent, vermifuge, emmenagogue, antiseptic, anaemi a, dyspepsia, antibacterial, dysentery, scurvy.	malic acid, vitamin C, tartaric acid, citric and malic acid, C-glycosyl flavonoid, vitexin, isovitexin, vitexin-2"-0-8-D-glucopyranoside.	Gunasegaran (1992)
11. *Sida acuta* (Malvaceae)	anthelminthic, abortifacient, abscesses, antipyretic, sedative, astringent, conjunctivitis.	cryptolepine, vasicine.	Morton(1981) Rao *et al* (1989)
12. *Zanthoxylum xanthoxyloides* (Rutaceae)	ulcer, syphilis, fever, poultice, conjunctivitis, laxative, anaemia, diarrhoea, **antimicrobial, cough (whooping)**	benzoic acid, derivatives, fagaridine, chelerythrine, skimmianine , **dihydrochelerythrine, arterine.**	Ayitey-Smith (1989) Ampofo(1983) Torto *et al* (1969)

Apetorgbor (1991) used aqueous and methanol extracts for hisantimicrobial studies on 19 test plants. He did not examine the effect of these extracts on the sclerotia production of S. *rolfsii* after treatment with the extracts. Foreasy comparison in this project aqueous and methanol extracts of the listed plants were used in testing for their antifungal activity on vegetative growth of *S. rolfsii* treated with the extracts. The economic importance of the two pathogens in Ghana cannot be overemphasised. *S. rolfsii* is the most frequently encountered fruit rot pathogen of tomato *(Lycoper si cum esculentum* Mill) in Ghana (Leather, 1959). It has been estimated that 30 percent of fruit losses is due to this fungus (Addison and Chona, 1971). S. rolfsii causes wilt of tobacco *(Nicotiana tabacum L.,* wilt of tomato, *(Lycopersicum escultentum),* bulb rot in onion *(Allium cepa L .) ,* wilt of groundnut *(Arachis hypogea L),* potato *(Solanum tuberosum L*) and cocoyam *(Xanthosoma sagitifolium)* (Leather, 1959). Weber (1931) indeed listed about 180 host species of this fungus including ferns, 8 monocotyledons and 42 dicotyledonous families.

Helminthosporium is a serious pathogen in Ghana causing leaf spot in maize *{Zea mays),* para rubber *(Hevea brasiliensis),* sorghum *(Sorghum vulgare),* sugar cane *(Saccharum officinarum)* and yam *(Dioscorea alata)* (Clerk, 1974; Vidhyasekran *et al.* 1985). Effect of the aqueous and methanol extract on some aspects of the physiology of *S. rolfsii* and *Helminthosporium sp.* are reported in this thesis. In the concluding chapter, the list of test fungi is extended to include *Aspergillus flavus* (producing aflatoxin in grain products), *Fusarium oxysporium* (causing wilt of tomato, banana, sweet potato and pears) and *A. niger* which attacks *Allium ascalonicum, Arachis hypogea, Butyrospermum*

parkii, Cocos nucifera, Elaeis guineense and Theobroma cacao (Piening, 1962).

MATERIALS AND GENERAL METHODS

1. MATERIALS

The isolates of *Sclerotium rolfsii*, Sacc., *Fusarium oxysporium*, *Helminthosporium sp.*, *Aspergillus niger* van Tieghem and *Aspergillus flavus* Link used in these investigations were obtained from stock culture collection of the Botany Department, University of Ghana, Legon. *S.rolfsii* and *F.oxysporium* were originally isolated from naturally infected tomato *(Lycopersicum esculentum* Mill) fruit. *A.niger*, *A.flavus* and *Helminthosporium* were isolated from phylloplane of cultivated okra (*Abelmoschus esculentum).* The 12 plants used are listed in Table IB.

2. METHODS

(a) Maintenance of stock culture

Stock cultures of the fungi were maintained on slants of Potato Dextrose Agar (PDA) . They were incubated at 28°C and subcultured every two weeks.

(b) Preparation of plant extracts.

Both water and methanol extracts were prepared from the desired plant parts indicated at the appropriated places in the text.In each instance 4 0g of sun-dried plant part was used to prepare a litre of stock solution. Plant part was blended using a " Moulinex Blender" Mill 2 and then strained. The supernatant liquid was filtered by vacuum suction pump to

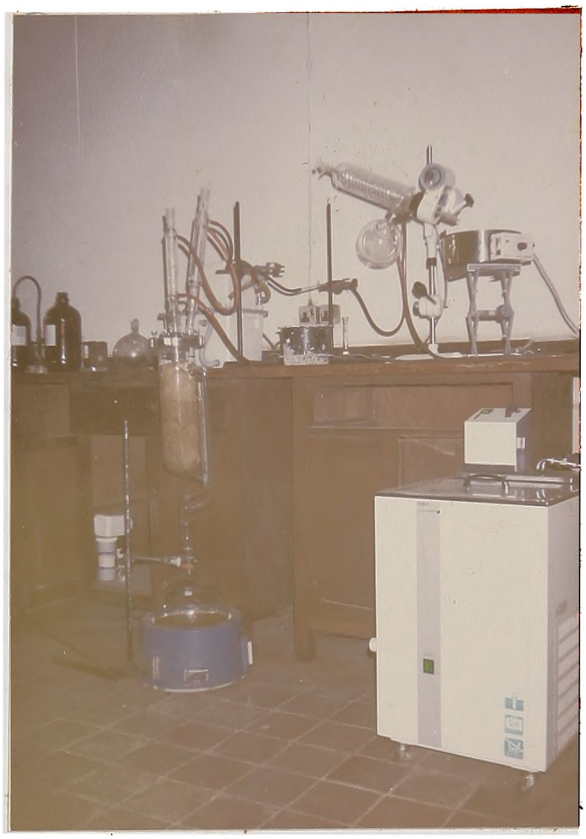


Plate 1. Photograph showing the apparatus used in the preparation of the methanol extracts of the test plants.

TABLE IB

List of plant species and their families used in these investigations..

Plant Species	Family.
Abrus precatorius Linn.	Papilionaceae
Alternanthera pungens (repens) Linn	Amaranthaceae
Azadirachta indica A Juss	Meliaceae
Boerhavia diffusa Linn	Nyctaginaceae
Cassia alata Linn.	Caesalpinaceae
Catharanthus roseus G Don.	Apocynaceae
Clausena anisata (Willd) Hook.f.ex.Benth	Rutaceae
Desmodium triflorum Linn.	Papilionceae
Mitragyna inermis (Willd) O.Kuntz	Rubiaceae
Oxalis corniculata Linn.	Oxalidaceae
Sida acuta Burm.f.	Malvaceae
Zanthoxylum xanthoxyloides Lam	Rutaceae

obtain a clearer solution. Methanol extract of the plant species was obtained using the Soxhlet extraction (Plate 1). The extract was then evaporated to dryness using the Rotary vacuum evaporator (Eyela Model). The resulting residue was re-suspended in one litre distilled water for immediate use and the rest stored in a refrigerator at 5°C till needed.

(c) Preparation of media.

(i) Synthetic medium.

Both liquid and solid media were used. The liquid medium composed of lOg glucose; lg yeast extract; 0.5 KH_2P0_4 (Potassium dihydrogen orthophosphate); O.OOlg (trace) of $FeCl_2$ (ferric chloride) and 1,000ml distilled water. About 20g of agar was added to obtain a solid medium.

(ii) Potato Dextrose Agar (PDA).

About 200g of peeled potato were boiled in 500ml of distilled water strained and made up to 1,000ml; 20g dextrose (glucose) and 2 0g agar were added.

(d) Solid medium.

The aqueous (water) or alcohol plant extract, were used in amending basal synthetic medium to obtain solid agar media of concentration, undiluted 1:1, 1:2 and l:5v/v dilution of the extracts. About 2 0ml of the extract of approximate dilution was poured into 9.0cm diameter sterile petri plates. The petri plates were then inoculated at the centre of the plates

with 3mm discs of the mycelium taken from the advancing edge of 5 day old fungal culture. In the case of *A.niger* and *A. flavus* the plate was inverted and the disc used in touching the surface of the agar. There were 4 replicates for each dilution level. The plates were incubated at 2 8°C for up to 7 days.

(e) Assessment of vegetative growth on solid medium.

Vegetative growth was assessed by measuring growth along two diameters drawn on the bottom of the petri plates. Readings were taken daily for *Helminthosporium, A.niger, A. flavus, F.oxysporium* and *S.rolfsii.*

(f) Assessment of Sclerotia production by mycelium of *S.rolfsii*.

The culture of *S. rolfsii* growing in basal medium amended with varying concentrations (undiluted, 1:1,1:2,1:5v/v) of plant extract was left to grow for 14 days in order to allow possible sclerotia formation. At the end of the incubation period,the number of sclerotia formed in each treatment (control, 1:1, 1:2, 1:5V/V) were counted. The diameters of the resulting sclerotia were also measured.

(g) Preparation of liquid medium.

The basal medium was amended with aqueous or methanol extract of plant to obtain 1:1, 1:2, l:5v/v dilution of the extract.

Erlenmeyer flask (250ml) containing 30ml of appropriate dilution of the extract were plugged with non absorbent-cotton wool and sterilized at 121°C for 15 minutes. The flasks were then inoculated with 3mm discs of 5 day old culture of the appropriate fungus mentioned at the right places in the text. Flasks were incubated for 10 days at 30±2°C. There were 16 replicates for each dilution level. At the pre-determined incubation period of 2,4,8 and 10 days, four flasks were harvested, dry weight and pH were determined as outlined below.

(h) Assessment of vegetative growth by the oven dry weight method.

Vegetative growth in the liquid cultures was assessed by harvesting the mycelium at the end of the required incubation period. Whatman no.2 filter papers were put in an electrically heated oven at 75°C for 24h. The filter papers were then put into a desiccator and allowed to cool and then reweighed. Filter paper carrying mycelium of the fungus were put in an oven at 75°C for 24h before reweighing.

(i) pH measurement.

The pH of culture media were determined using an alpha 500 pH meter. Readings of pH were taken of autoclaved medium just before inoculation and at the end of each experiment pH of the supernatant liquid was estimated.

(j) Persistence of inhibitory effect of plant extract on vegetative growth of test fungi.

In order to find out whether *S. rolfsii* and *Helminthosporium sp* would recover from the depressing effect of methanol leaf extract of *C.alata*, 3mm discs of mycelium were buried in the undiluted leaf extract of *C.alata* for 1/2, 1, 2, 6, 12, 18, 24 and 48hrs.At the end of the desired incubation period, the mycelia were removed and washed thoroughly in three changes of distilled water and then used in inoculating 250ml Erlenmeyer flask containing 30ml of the liquid basal medium. There were 4 replicates for each treatment level. The flasks were incubated at 3 0+2 C for 8 days. Vegetative growth was assessed using the oven dry weight method.

(k) Incubation Period.

Incubation conditions varied with the experiment and it is stated at the appropriate places in the text.

(1) Chemicals.

All chemicals used in the preparation of media were either of the "Analar" or of the BDH (British Drug House) grade;both distributed by British Drug House, Poole, England. Erlenmeyer pyrex flasks (250ml capacity) each containing 30ml media were used for the broth cultures.

(m) Experimental Precautions.

Glassware were kept scrupulously clean. Those which had already been cleaned with water and detergent were rinsed several times with tap water and three times with distilled water and allowed to drain before use. Petri dishes used were sterilised at 160°C for 8h in an electrically heated oven. All media, beakers (250ml), measuring cylinders and other glassware were sterilised by autoclaving at 121°C for 15 minutes at a pressure of 1.05kg/cm.
Inoculating needles and loops as well as cork bores were flame-sterilised just before use.

The laminar flow cabinet in the inoculating room was cleaned with 5% dettol and switched on 30 minutes before use.

As far as possible spores of the same age were used in all experiments. Spores serving as inoculum for the experiment were always obtained from 5 days old culture.

Filter papers with harvested mycelium were conveyed to the balance room after drying in the oven in a desiccator to avoid absorption of moisture. Filter papers for harvesting of the mycelium were heated at 75°C for 24hrs and re-weighed prior to use.

(n) Statistical analysis.

Statistical analysis where necessary,was carried out on the data using the analysis of variance and Scheffe Multiple Range Test.Results are quoted as statistically significant at 5% ($P < 0.05$) level of significance.

EXPERIMENTAL PROCEDURE

A. VEGETATIVE GROWTH OF *S. ROLFSII* AND *HELMINTHOSPORIUM SP.* IN BROTH MEDIUM AMENDED WITH EXTRACTS OF PLANTS IN THE FAMILIES CAESALPINACEAE (CAESALPINOIDAE) AND PAPILIONACEAE (PAPILINOIDAE).

Plants contain many compounds that have been used in herbal therapy, inhibitory growth of microorganisms and as biopesticides (Van Latum and Gerrits, 1991). *S. rolfsii* and *Helminthosporium sp.* are of economic importance in the country. It was anticipated that extracts of selected plants in the families Caesalpinaceae (Tr. Caesalpinoidae) and Papilionaceae (Papilinoidae) namely *Abrus precatorius, Cassia alata and Desmodium triflorum* would be able to depress vegetative growth and sporulation of *S. rolfsii* and *Helminthosporium sp.* in vitro and the active ingredients could be used in biocontrol. Broth medium was amended with varying dilutions (1:1 to 1:5v/v) aqueous or methanol extracts of the plants and the flask were inoculated with 3mm discs of mycelium of the test fungi (see Materials and General Methods) . The oven dry weight method was used in assessing vegetative growth. Results obtained are presented in Figs 1 and 2 and in Appendices 1-6.

B.VEGETATIVE GROWTH OF *S. ROLFSII* AND *HELMINTHOSPORIUM SP.* ON AGAR AMENDED WITH EXTRACTS OF PLANTS IN THE FAMILIES CAESALPINACEAE (CAE SAL PI NO I DAE) AND PAPILIONACEAE (PAPILIONOIDAE).

There are instances where vegetative growth of the fungi in liquid medium was different from what obtained on agar (Held, 1955) . Furthermore, the depth of the medium and aeration may inf luence growth (Brancata and Golding, 1953) . The experiments in Chapter A were repeated, this time the plant extracts were amended with agar to obtain the various concentrations (1:1-1:5v/v). Vegetative growth of the test fungi was assessed along two diameters drawn on the bottom of the petri plates. Results obtained are presented in Tables 2 and 3 and Appendices 7-12.

C.PRODUCTION OF SCLEROTIA BY *S. ROLFSII* ON AGAR MEDIUM AMENDED WITH EXTRACTS OF PLANTS IN THE FAMILIES CAESALPINACEAE (CAESALPINOIDAE) AND PAPILIONACEAE (PAPILIONOIDAE).

The period of incubation for experiment in Chapter B was extended for 7 days to enable the mycelium growing on the amended media form sclerotia (if possible). The ability of the plant extracts in depressing sclerotia formation was evaluated by counting the number and dimensions of sclerotia formed in the different extracts. Results obtained are summarised in Table 4 and fig. 3. Statistical analyses are

presented in Appendices 13-16.

D.VEGETATIVE GROWTH OF *S. ROLFSII* AND *HELMINTHOSPORIUIM SP.* IN BROTH MEDIUM AMENDED WITH EXTRACTS OF PLANTS IN THE FAMILIES AMARANTHACEAE, MALVACEAE. NYCTAGINACEAE AND OXALIDACEAE.

Variation in depression of vegetative growth of *S. rolfsii* and *Helminthosporium sp.* in media amended with extracts of plants in the families Caesalpinaceae and Papilionaceae, necessitated the extension of the list of test plants to include members in the above-named families including some plants whose fungicidal and bacticidal actions have been demonstrated by Apetorgbor (1991). It was necessary to confine the nature of the extract to aqueous and methanol extracts in the first instance so that many plants can be covered using the same test fungi. The procedure adopted was same as in Chapter A and vegetative growth was assessed using the oven dry weight method. Figs. 4-7 show results obtained and statistical analyses of the results are summarised in Appendices 17-20.

E. VEGETATIVE GROWTH OF *S. ROLFSII* AND *HELMINTHOSPORIUM SP.* OF AGAR AMENDED WITH LEAF EXTRACTS OF PLANTS IN THE FAMILIES AMARANTHACEAE. MALVACEAE. NYCTAGINACEAE AND OXALIDACEAE.

The experiments in Chapter B were repeated on agar amended with the plant extracts to obtain varying concentrations

(1:1-1:5v/v dilution). Vegetative growth of the test fungi was assessed along two diameters drawn on the bottom of the petri plates. Results obtained are presented in Tables 5 and 6 and in Appendices 21-24.

F. SCLEROTIA PRODUCTION BY *S. ROLFSII* ON AGAR AMENDED WITH AQUEOUS AND METHANOL EXTRACTS OF PLANTS IN THE FAMILIES AMARANTHACEAE. MALVACEAE AND NYCTAGINACEAE AND OXALIDACEAE.

The extracts of the plants used in Chapters D and E depressed vegetative growth of *S. rolfsii*. One question of practical importance is whether the same extracts can concurrently depress and even prevent sclerotia formation. Both aqueous and methanol of dry leaf extracts of *Sida acuta* (Malvaceae) , *Boerhavia diffusa* (Nyctaginaceae), *Oxalis corniculata* (Oxalidaceae) and *Alternanthera repens* (Amaranthaceae) were used. The number of sclerotia as well as their dimensions were determined. Table 7 and Fig. 8 show results obtained and statistical analyses of the data are summarised in Appendices 25-28.

G. VEGETATIVE GROWTH OF MYCELIUM OF *S. ROLFSII* AND *HELMINTHOSPORIUM SP.* ON AGAR MEDIUM AMENDED WITH EXTRACTS OF PLANTS IN THE FAMILIES MELIACEAE AND RUTACEAE.

The aqueous and methanol dry leaf extracts of *A. indica, C. anisata* and *Z. xanthoxyloides* were tested for their ability to depress vegetative growth of *S. rolfsii* and

Helminthosporium sp. in broth amended with varying dilutions of the plant extracts. The methods employed and the assessment of vegetative growth were the same as in Chapters B and D. Results obtained are presented in Tables 8 and 9 and Appendices 2 9-33.

H. VEGETATIVE GROWTH OF *S. ROLFSII AND HELMINTHOSPORIUM SP.* IN BROTH MEDIUM AMENDED WITH EXTRACTS OF PLANTS IN THE FAMILIES MELIACEAE AND RUTACEAE.

The experiments in Chapter G were repeated this time agar was excluded from the medium. The rationale was to establish any differences in growth rate on agar and in liquid broth medium. Dry weight was assessed using the oven dry weight method. Figs. 9 and 10 summarise results obtained. Statistical analyses of the results are presented in Appendices 34a-c.

I. PRODUCTION OF SCLEROTIA ON AGAR AMENDED WITH EXTRACTS OF PLANTS IN THE FAMILIES MELIACEAE AND RUTACEAE.

The experiment in Chapter G was repeated. The production of sclerotia ensured the prolonged survival of the pathogen *S. rolfsii* under adverse environmental conditions. If indeed, the extracts from plants in the families are able to offset sclerotia formation, they could be employed to attack this phase in the life cycle of the fungus. The incubation period was extended to fourteen days to allow the fungus to form

sclerotia (if possible). The number formed as well as their dimension were noted. Results are summarised in Table 10, Fig.11 and Appendices 35-38 represent statistical analyses of data obtained.

J. VEGETATIVE GROWTH OF *S. ROLFSII AND HELMINTHOSPORIUM SP.* ON AGAR MEDIUM AMENDED WITH EXTRACT OF PLANTS IN THE FAMILIES APOCYNACEAE AND RUBIACEAE.

The list of test plants was extended to include members of the above-named families which are well known for their use in herbal therapy and antimicrobial activity elsewhere but not in Ghana. The methods of assessment of vegetative growth have been described in the Materials and General Methods Section. Results obtained are presented in Tables 11-14.

K. VEGETATIVE GROWTH OF *S. ROLFSII AND HELMINTHOSPORIUM SP.* IN BROTH MEDIUM AMENDED WITH LEAF EXTRACT OF PLANTS IN THE FAMILIES APOCYNACEAE AND RUBIACEAE.

In some instances depression of vegetative growth on agar was severer than in broth whilst in others, no significant differences were observed. (Chapters A-J) . Since there was no general rule, it was expedient to repeat experiments in Chapter I in broth medium to confirm or otherwise the data obtained. The experimental set up and methods used are described in the Materials and General Methods Section. Figs. 12 and 13 data obtained and statistical analyses of data are

presented in Tables 15 and *16.*

L. PRODUCTION OF SCLEROTIA IN MEDIUM AMENDED WITH LEAF EXTRACTS OF PLANTS IN THE FAMILIES APOCYNACEAE AND RUBIACEAE.

The experimental design and reason for investigations in this Chapter were the same as for Chapters B, E and H. The rational is to extend the list of plants whose extracts adversely influence sclerotia production by *S. rolfsii.* Results obtained are summarised in Table 17, fig.14 and statistical analyses presented in Appendices 39 and 40.

M. VEGETATIVE GROWTH OF *S. ROLFSII* AND *HELMINTHOSPORIUM SP.* BURIED IN METHANOL EXTRACT OF CASSIS ALATA FOR VARYING PERIODS.

An information of practical importance is the period of minimum contact with the extracts required to permanently impair vegetative growth of the test fungi. The shorter this period, the more potent the active ingredients. Mycelial disc of the test fungi were left buried in the liquid methanol extract of C. alata for periods 30 minutes up to 48hrs before transfer into extract free medium. The methanol extract of *C. alata* has shown some promise from results of the previous investigations in this thesis and was chosen to ascertain its efficacy. Results obtained are represented in Figs.15-18 and Appendices 41-47.

N. EFFECT OF LEAF EXTRACTS OF *C. ALATA* AND A. *PRECATORIUS* ON VEGETATIVE GROWTH OF *A. NIGER. A. FLAVUS* AND *F. OXYSPORIUM.*

In this concluding Chapter of the series of investigations the fungistatic potential of *C. alata* and *A. precatorius* was tested on three potentially pathogenic fungi namely *A. niger, A. flavus* and *F. oxysporium.* Growth rate on agar amended with both aqueous and methanol extracts of the test plants was assessed along two diameters. Results obtained are presented in Figs. 19 and 20, and in Appendices 48 and 49.

RESULTS

A .VEGETATIVE GROWTH OF *S. ROLFSII* AND *HELMINTHOSPORIUM SP.* IN BROTH MEDIUM AMENDED WITH EXTRACTS OF PLANTS IN THE FAMILIES CAESALPINACEAE AND PAPILIONACEAE.

Results obtained are presented in Appendices 1-6 and Figs.1 and 2

Aqueous Extract.

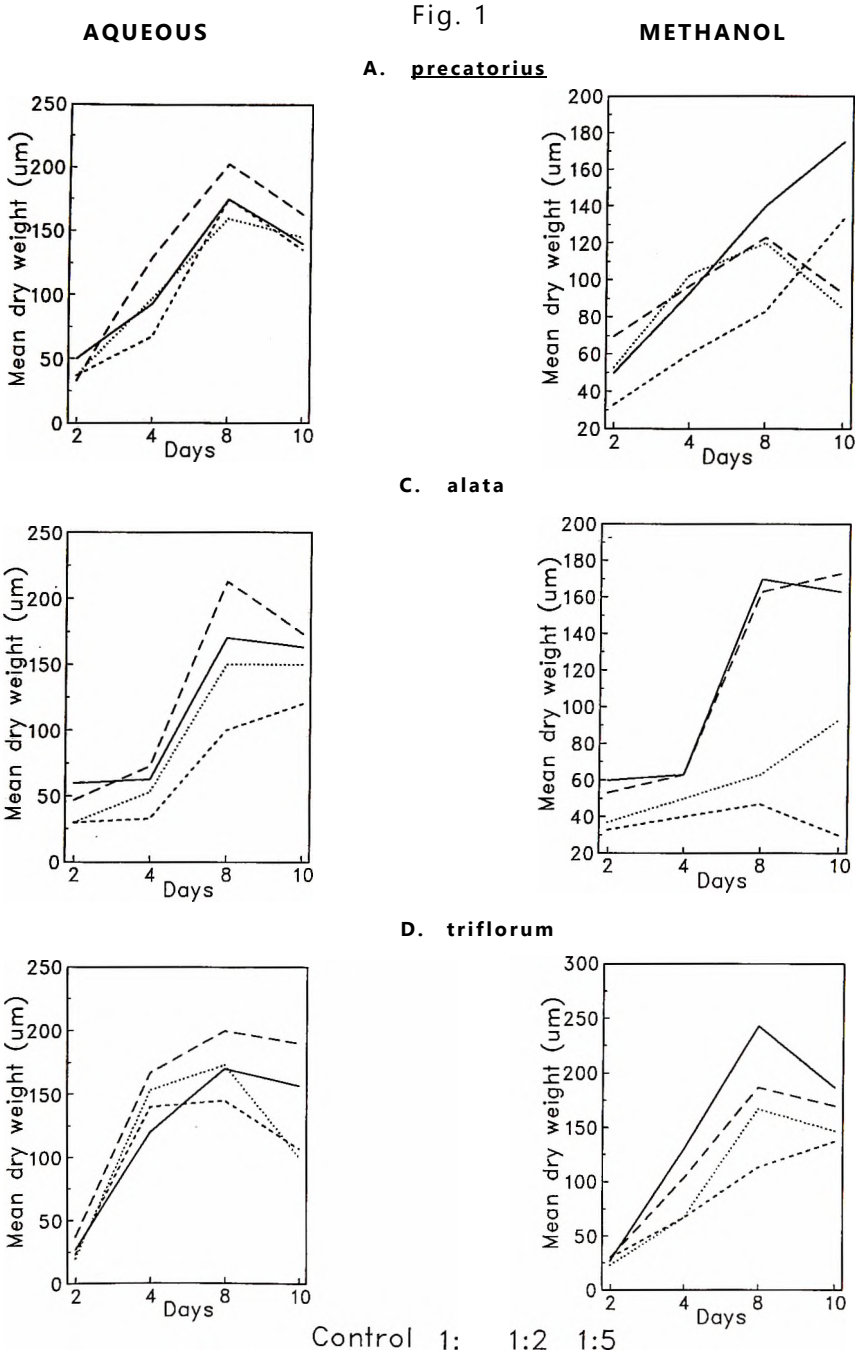
There was statistical difference (P s 0.05) between vegetative growth of the two test fungi in the media amended with the aqueous extracts of *A. precatorius, C. alata* and *D. triflorum* (Appendices 1-6). Inhibition of growth of the test fungi in the plant extract was severer on *Helminthosporium sp.* than on *S. rolfsii* (Appendices 1-6). The effect was severer at higher concentrations of the extract and was gradually removed with increasing dilution.

Methanol Extract.

The effect of the methanol extracts on the test fungi was variable. There was a significant effect (P s 0.05) of the alcohol extracts of *C. alata* and *D. triflorum* on the vegetative growth of the test fungi (Appendices 1-6). For example, medium amended with 1:1, 1:2 and 1:5v/v of *C. alata* depressed vegetative growth by 75.79 percent, 63.25 and 14.83 percent respectively; (Figs 1 and 2) same concentration of *D. triflorum* depressed vegetative growth

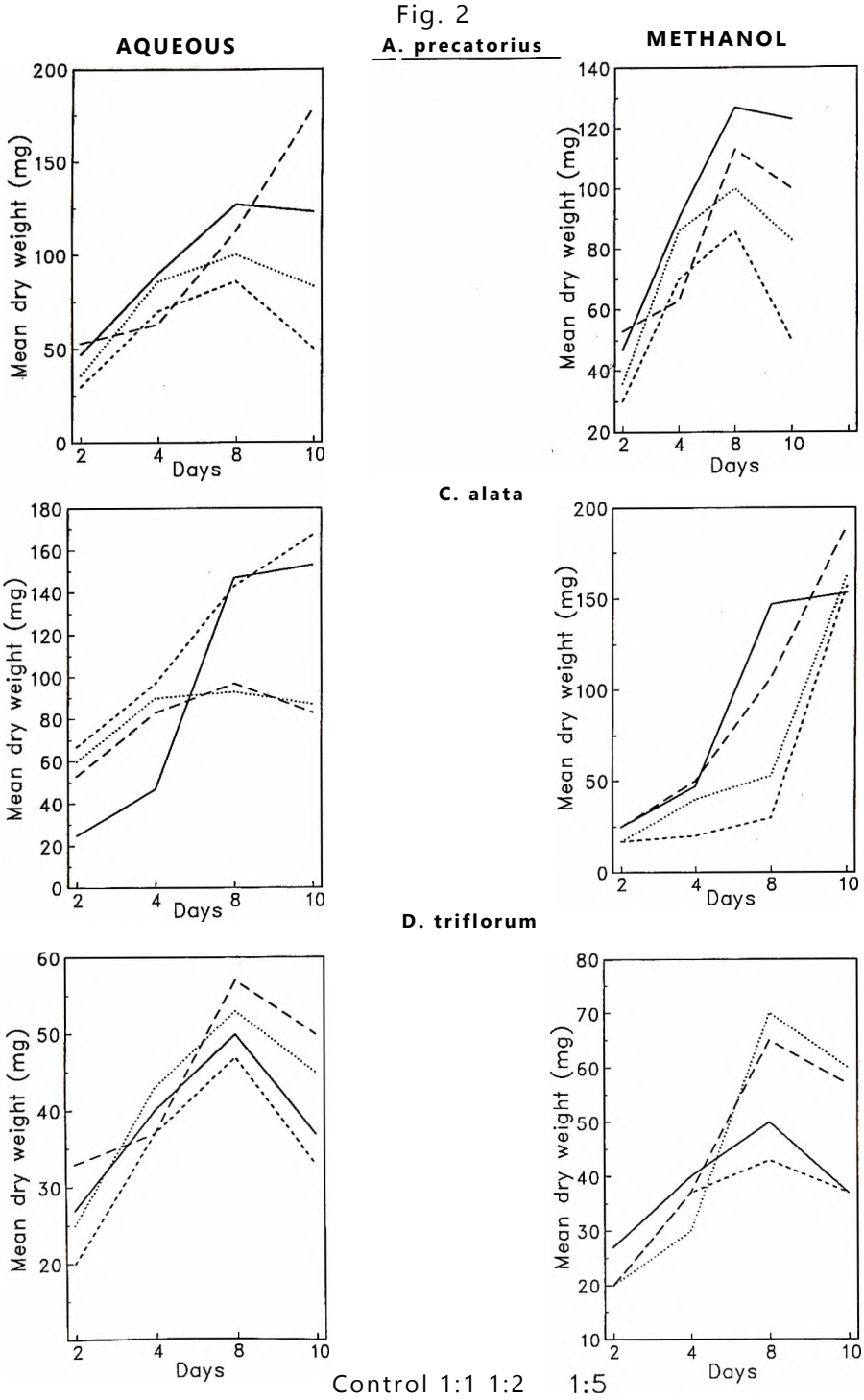
by 46.89, 19.27 and 16.71 respectively (Figs 1 and 2). Extract dilution of 1:1v/v of *A. precatorius* depressed growth of the test fungi by 45.27 percent (Figs. 1 and 2).

Fig. 1



Vegetative growth of _S. rolfsii in liquid medium amended with aqueous and methanol extracts of A. precatorius, C. alata and D. triflorum.

Fig. 2



Vegetative growth of Helminthosporium sp. in liquid medium amended with aqueous and methanol extracts of indicated plants.

B. VEGETATIVE GROWTH OF *S. ROLFSII* AND *HELMINTHOSPORIUM SP.* ON SOLID MEDIUM AMENDED WITH EXTRACTS OF PLANTS IN THE FAMILY CAESALPINACEAE AND PAPILIONACEAE.

Results obtained are summarised in Tables 2 and 3, Appendices7-12, Plates 3 and 4. The aqueous and methanol extracts of C. *alata, D. triflorum* and *A. precatorius* significantly (P <s 0.01) depressed the vegetative growth of the two test fungi. In all instances vegetative growth of *Helminthosporium sp.* was significantly (P s 0.05) depressed than that of *S. rolfsii* (Appendices 7b, 8b, 9b, 10b, lib and 12b). The inhibitory effect was gradually removed with dilution of the extract. In some instances, methanol extract was more effective than the aqueous extract of the same plant. For example, methanol extract (l:lv/v dilution) of *A. precatorius* depressed vegetative growth by 56.49 percent whilst the aqueous extract (l:lv/v dilution) depressed vegetative growth by 30.88 percent.

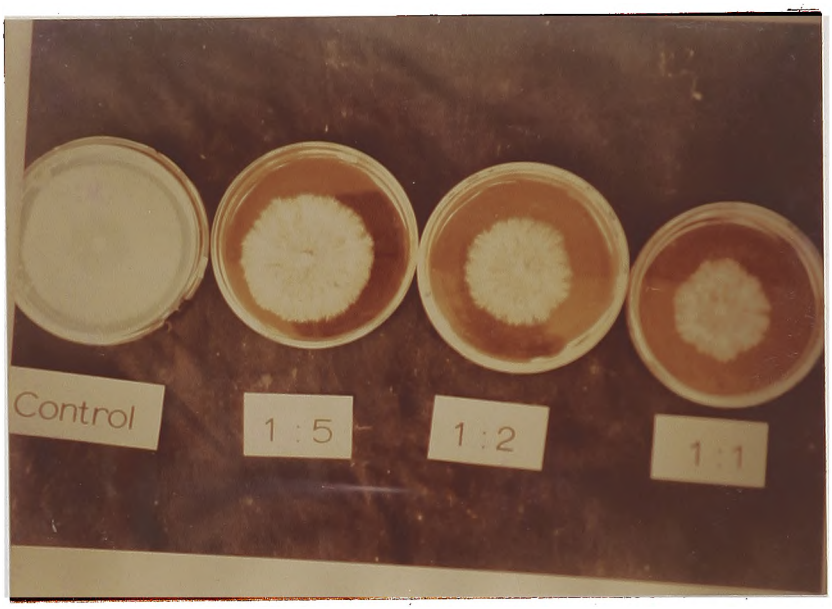


Plate 2. Radial growth of S. rolfsii at 28°C for 4 days on agar medium amended with methanol dry leaf extract of C. alata.

(Note the severer depression of growth at 1:1 and 1:2v/v dilutions).

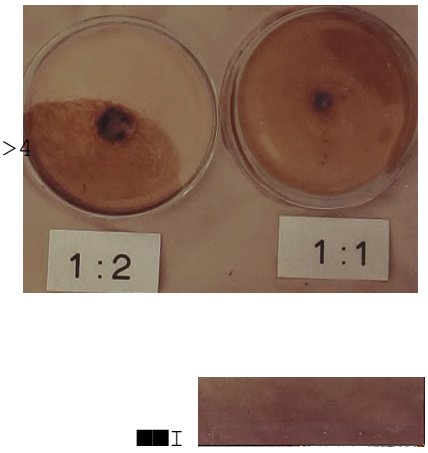


Plate 3 Radial growth of Helminthosporium SP. at 2 8°C for 4 days on agar medium amended with indicated dilutions of methanol dry leaf extract of C. alata.

Table 2

Vegetative growth of Sclerotia rolfsii at 28°C on agar medium amended with varying dilutions of extracts of indicated plants.

Plant species	Type of extract	Dilution ratio (v/v)	Mean Diameter of culture (mm) after (days) 2 3 4 5 6 7		
Abrus precatorius	Aqueous	Control	10.50 27.10 52.75 77.2090.00	*	*
		.1 : 1	- 11.80 29.50 45.7063.00	*	*
		1 : 2	- 17.10 39.50 58.0081.75	*	*
		1:5	- 21.20 42.25 61.3287.00	*	*
	Methanol	Control	8.00 37.25 63.12 85.0090.00	*	*
		1:1	— 17,75 44.00 59.5090.00	*	*
		1:2	18.25 46.00 66.5090.00	*	*
		1:5	- 20.50 48.00 66.5090.00	*	*
Cassia alata (Caesalpinaceae)	Aqueous	Control	- 10.00 31.00 62.0090.00	*	*
		1:1	- - 23.00 45.0071.50	*	*
		1:2	- 10.00 32.00 53.5077.00	*	*
		1:5	— 9.50 30.30 56.5083.50	♦	*
	Methanol	Control	11.00 31.25 51.00 81.0090.00*	90.00	*
		1:1	8.50 8.50 11.50 13.50	15.50	*
		1:2	- 8.75 17.50 19.2523.00	27.25	*
		1:5	- 1 1.75 23.25 30.7535.50	40.25	*
Desmodium triflorum (Papilionaceae)	Aqueous	Control	18.25 38.80 57.50 78.00 *	*	*
		1:1	20.00 40.00 67.50 90.00 *	*	*
		1:2	20.75 40.30 68.30 90.00 *	*	*
		1:5	21.00 42.80 69.50 90.00 *	*	*
	Methanol	Control	13.80 35.00 72.80 90.00 *	*	*
		1:1	11.30 24.50 48.80 69.00 »	*	*
		1:2	13.30 28.50 52.30 73.80 *	*	*
		1:5	13.50 30.80 56.50 80.80 *	*	*

— No growth.
*** Reading discontinued.**

Table 3

Vegetative growth of Helminthosporium sp. at 28°C on agar medium amended with varying dilutions of extracts of indicated plants.

Plant species	Type of extract	Dilution ratio (v/v)	Mean Diameter of culture (mm) after (days) 1	2	3	4	5	6	7
Abrus precatorius (Papilionaceae)	Aqueous	Control	9.00	19.75	28.80	36.50	4 1.50	52.50	66.00
		1:1	-	8.75	9.25	10.00	13.00	15.00	15.00
		1:2	-	11.00	15.25	20.75	23.50	25.00	27.50
		1:5	7.00	15.00	20.75	26.75	30.25	34.50	36.00
	Methanol	Control	7.750	14.75	25.00	3 1.50	39.50	48.50	57.50
		1:1	-			9.00	11.00	18.00	22.15 25.25
		1:2	-	9.00	12.00	14.82	26.50	32.00	32.00
		1:5	7.50	12.75	23.00	26.50	32.00	36.65	41.10
Cassia alata (Caesalpinaceae)	Aqueous	Control	9.00	19.75	28.80	36.50	4 1.50	52.50	66.00
		1:1		6.25	8.50	10.50	14.00	18.75	22.40
		1:2	-	8.25	13.00	19.00	23.75	30.75	33.50
		1:5	7.0	12.00	17.25	22.00	27.25	30.00	32.00
	Methanol	Control	8.00	14.75	18.50	24.75	32.00	42.25	52.00
		1:1	6.50	6.50	6.75	8.00	9.00	9.00	9.00
		1:2	7.00	8.00	8.00	9.50	10.00	10.50	11.25
		1:5	6.50	9.00	9.00	9.00	9.25	9.75	10.25
Desmodium triflorum (Papilionaceae)	Aqueous	Control	10.75	21.25	31.00	42.25	53.25	63.75	52.00
		1:1	10.00	17.25	2 1.00	27.25	33.50	38.00	47.75
		1:2	9.75	15.75	25.00	31.50	37.00	44.75	52.75
		1:5	1 1.50	18.50	29.25	38.25	49.50	58.75	66.50
	Methanol	Control	8.00	14.75	18.50	25.75	32.00	42.25	52.00
		1:1	11.00	18.00	19.50	20.50	25.50	26.75	32.75
		1:2	10.50	17.50	20.50	24.25	27.00	29.00	37.75
		1:5	1 1.50	18.00	21.25	24.50	30.25	33.00	36.50

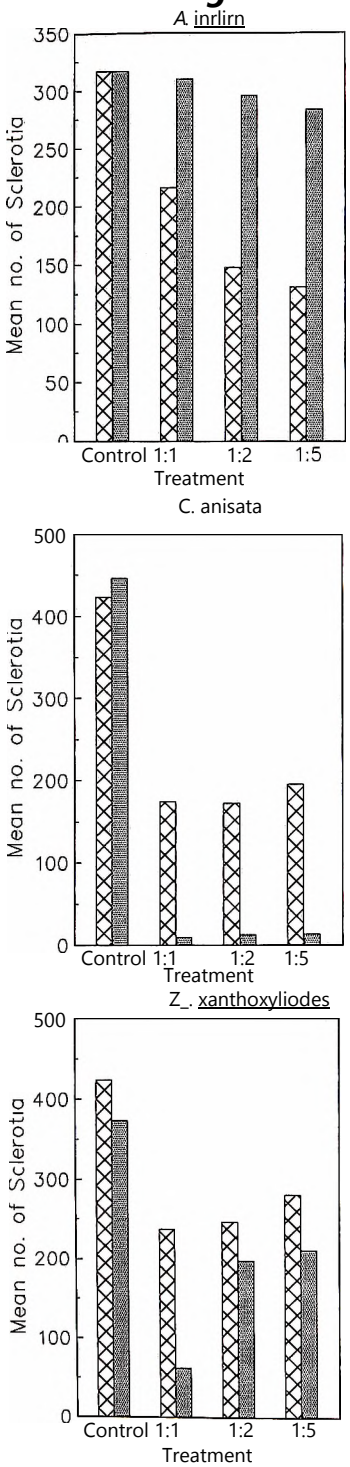
— No growth.

C. PRODUCTION OF SCLEROTIA BY *S. ROLFSII* ON AGAR MEDIUM AMENDED WITH EXTRACTS OF PLANTS IN THE FAMILIES CAESALPINACEAE AND PAPILIONACEAE.

Results obtained are summarised in Fig 3; Table 4 and Appendices 13-16. Both aqueou and ethanol extracts of the leaves of *A.precatorius*, *C. alata* and *D.triflorum* depressed sclerotia production to different extent (Table 4). For example,in the aqueous extract the depression in sclerotia production by *A.precatorius* was significantly higher (P s 0.05) than what existed in the presence of *C.alata* or *D.triflorum* (Appendices 13a). Where fewer sclerotia were formed in the higher concentrations (1:1, 1:2v/v dilutions) these sclerotia were significantly (P s 0.05) larger in diameter than the control (Appendix 15a). The inhibitory effect of the extracts on sclerotia production was gradually removed although not completely with dilution (up to 1:5v/v). Methanol extract of the leaves of the test plants also depressed sclerotia production (Table 4; Fig 3). Sizes of surviving sclerotia produced on agar amended with ethanol extract of *A.precatorius* did not differ significantly (P =s 0.05) from that of C. *alata.* On the contrary, sclerotia produced on agar amended with ethanol extract of *D. triflorum* was significantly (P =s 0.05) the smallest in size (Appendices 16a and 16b). Thus, the aqueous and alcohol extracts of *A.precatorius* and *C.alata*

have the potential of depressing sclerotia production by *S.rolfsii*. However, the fewer sclerotia formed tended to be larger. The inhibitory effect was also gradually removed with increasing dilution of the extracts.

Fig 3



Sclerotia production by S. rolfsii on agar amended with indicated plant extracts.

Table 4

Effect of aqueous and methanol extracts of indicated plants on sclerotia production by S. rolfsii on agar after 14 days at 28 C

Plant species	Nature of extract	Dilution of extract (v/v)	No. of sclerotia Mean + S.E		% depression Dia.	of sclerotia (mrr Mean + S.E	
Abrus precatorius iraDiiionaceaei	Aaueous	1:1	9	1.29	97.61	62.00	4.43
		1:2	13	1.22	95.60	77.63	2.54
		1:5	28	1.00	91.17	82.42	2.10
		Control	317	1.47	-	90.85	1.21
	Methanol	1:1	9	1.00	97. 17	70.59	0.56
		1:2	15	2.63	95.28	72.94	2.62
		1:5	28	1.41	91.19	78.67	2.68
		Control	318	4.16	-	90.85	1.21
Cassia alata (Caesalpinaceae)	Aqueous	1:1	52	1.82	79.69	76.71	3.18
		1:2	70	2.58	72.66	71.04	5.00
		1:5	153	26.78	40.23	75.67	2.21
		Control	256	4.40	-	85.79	1.30
	Methanol	1:1	1	0.29	99.66	87.62	1.87
		1:2	2	0.65	99.32	67.00	2.85
		1:5	5	1.19	98.29	73.33	2.36
		Control	292	4.13	-	87.92	2.75
Desmodium triflorum (Papilionaceae)	Aqueous	1:1	143	3.1 1	54.89	80.65	2.05
		1:2	125	8.42	60.57	88.85	1.47
		1:5	1 13	3.1 1	64.35	88.90	0.53
		Control	317	9.47	-	85.60	1.9 1
	Methanol	1:1	310	7.36	3.13	73.80	0.35
		1:2	408	35.42	NDO	67.88	2.39
		1:5	408	5.34	NDO	66.37	0.46
		Control	320	4.56	-	78.48	1.63

NDO- No depression occurred.

D. VEGETATIVE GROWTH OF *S. ROLFSII* AND *HELMINTHOSPORIUM* SP- IN BROTH MEDIUM AMENDED WITH EXTRACTS OF PLANTS IN THE FAMILIES AMARANTHACEAE. MALVACEAE. NYCTAGINACEAE AND OXALIDACEAE.

Results obtained are summarised below:

Aqueous extract.

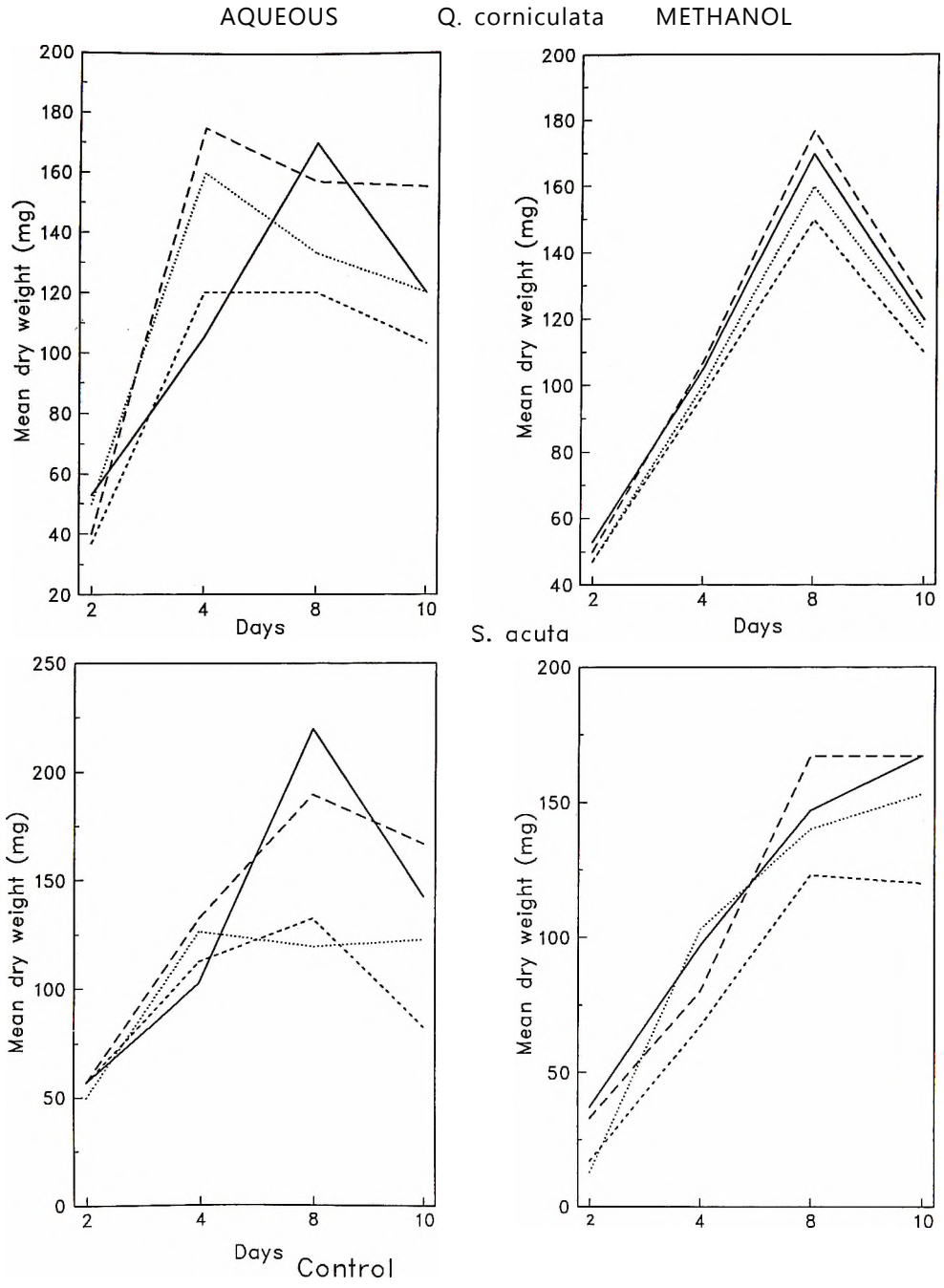
The aqueous leaf extracts marginally depressed vegetative growth of the test fungi (Figs 4-7). For example, aqueous extract of *S. acuta* of dilution 1:1, 1:2 and l:5v/v depressed vegetative growth by 38.72, 30.79 and 10.60 percent respectively. The low inhibitory effect was severer on *Helminthosporium sp.* than on *S. rolfsii.* Generally, the inhibitory effect was removed with increasing dilution of the extracts such that growth in the l:5v/v approximated that of the control (Figs 4-7).

Methanol extract.

Depression in the vegetative growth of the test fungi by methanol extract of the test plants was nil to marginal in most instances. For example, nutrient media amended with methanol extracts of O. *corniculata* did not significantly (P ^ 0.05) suppress vegetative growth of *S. rolfsii* and *Helminthosporium sp.*(Appendices 17a,19a and 20a). This

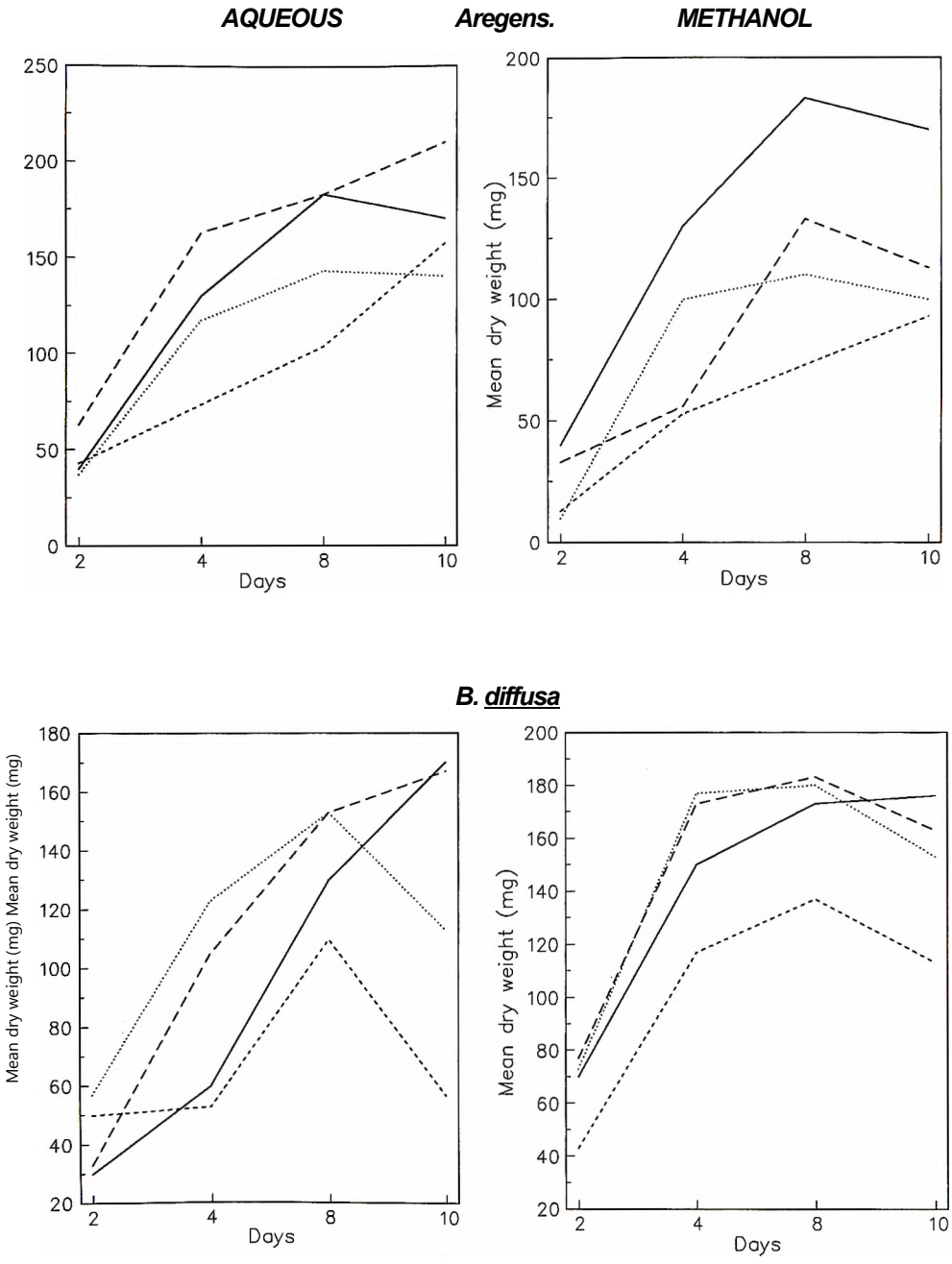
observation connotes that the extracts do not seem to exert any appreciable effect on the fungi. However media amended with methanol extracts of *A. repens* of concentration 1:1, 1:1 and 1:5v/v inhibited vegetative growth of the test fungi by 35.04. 29.06 and 19.66 percent respectively (Fig 4-7). The inhibitory effect was gradually removed by increasing dilution of the extract.

Fig.4



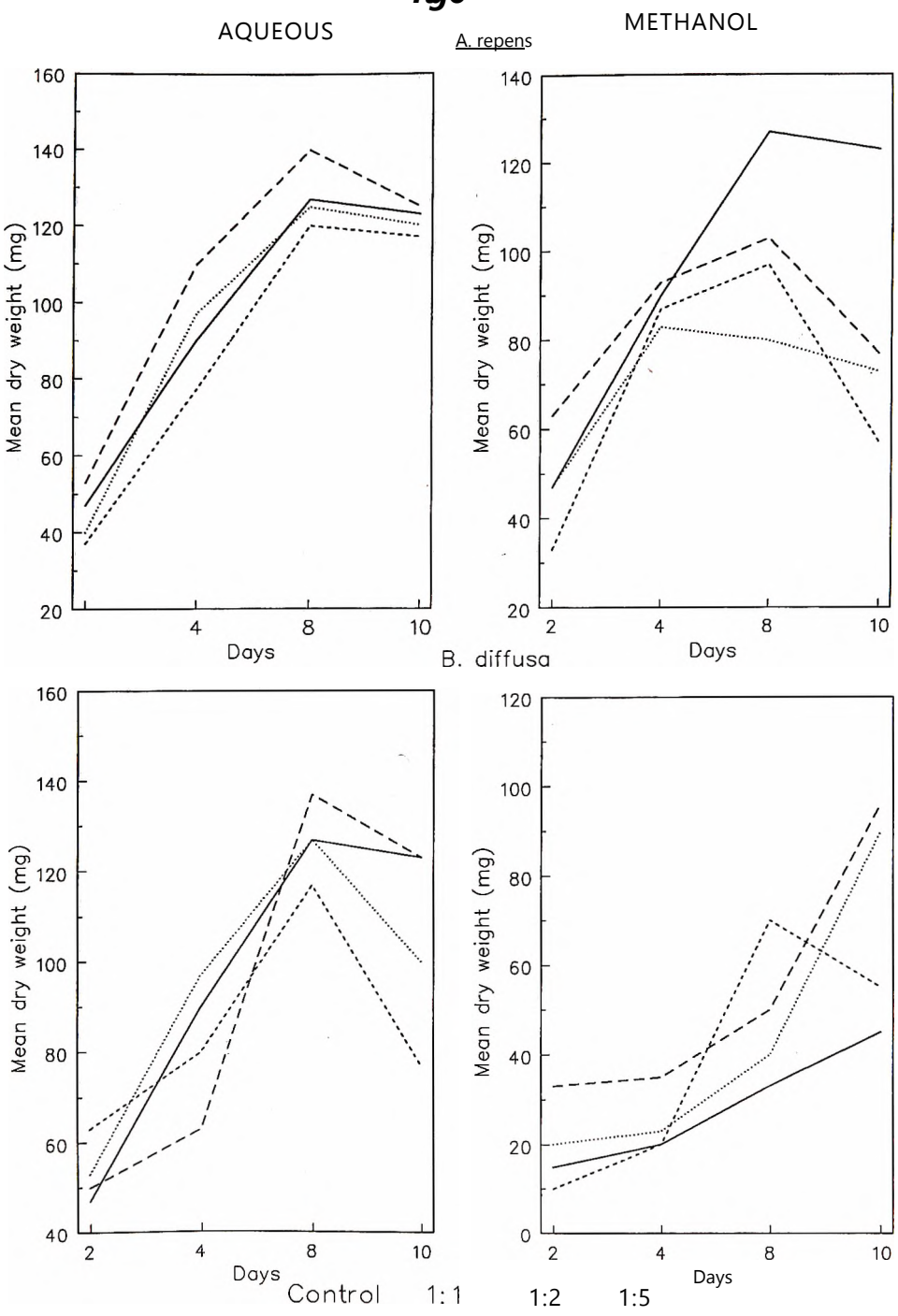
Vegetative growth of S^ rolfsii at 28 C in liquid medium amended with aqueous and methanol extracts of 0. corniculata and S. acuta.

Fig5



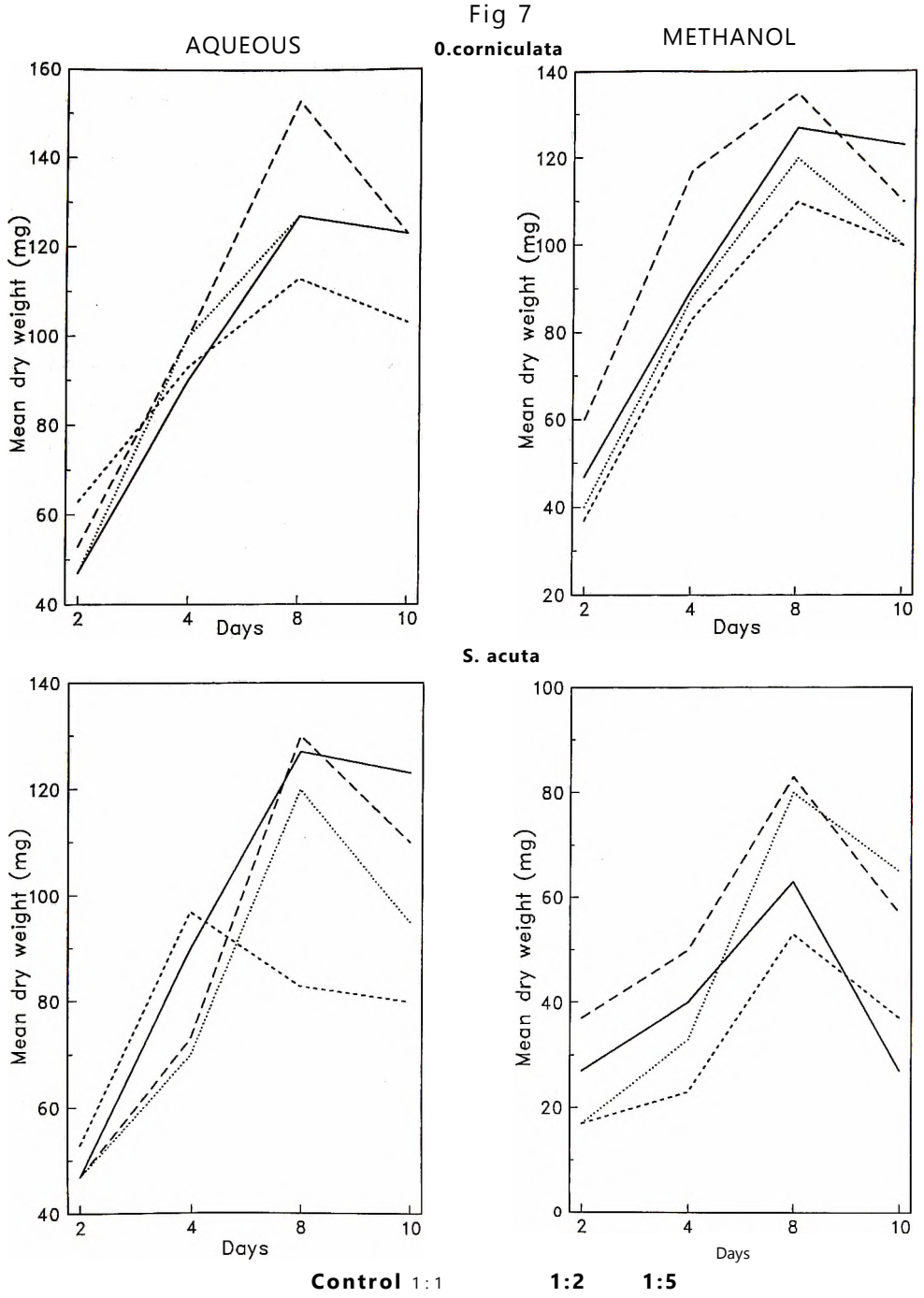
Vegetative growth of rolfsii at 28°C in liquid medium amended with aqueous and methanol extracts of A. precatorius and B. diffusa.

Fig6



Vegetative growth of Helminthosporium sp. at 28°C in liquid medium amended with aqueous and methanol extracts of /V_ repens and E3. diffusa.

Fig 7



Vegetative growth of Helminthosporium sp.in liquid medium amended with aqueous and methanol extracts of 0^ corniculata and S. acuta.

E. VEGETATIVE GROWTH OF *S. ROLFSII* AND *HELMINTHOSPORIUM SP.* ON AGAR MEDIUM AMENDED WITH LEAF EXTRACTS OF PLANTS IN THE FAMILIES AMARANTHACEAE, MALVACEAE. NYCTAGINACEAE AND OXALIDACEAE.

Results obtained did not differ significantly from what existed in the liquid medium. In both media amended with aqueous and methanol extracts of the test plants depression in vegetative growth by the highest concentration (l:lv/v) did not exceed 65.35 percent (Tables 5 and 6,Plate 4). Further dilution rather enhanced vegetative growth of *S. rolfsii* and *Helminthosporium sp.*. The effect of the inhibitory principles in the plants was severer on *Helminthosporium sp.* in most instances (Appendices 21-24).

Table 5

Vegetative growth of Sclerotium rolfsii at 28°C on agar medium amended with varying dilutions of extracts of indicated plants.

Plant species	Type of extract	Dilution raTio (v/v) ,	Mean Diameter of culture (mm) after day 1	2	3	4	5	6	7
Alternanthera repens (Amaranthaceae)	Aqueous	Control	17.00	44.00	77.30	90.00	*	*	*
		1:1	19.50	45.80	79.80	90.00	*	*	*
		1:2	20.30	46.80	79.80	90.00	*	*	*
		1:5	20.50	49.00	81.50	90.00	*	*	*
	Methanol	Control	1 1.00	31.25	51.00	81.00	90.00	90.00	
		1:1	9.00	10.00	21.75	30.50	31.50	38.00	42.25
		1:2	9.00	16.75	21.00	29.00	35.25	40.25	44.00
		1:5	10.00	30.75	39.00	43.00	48.00	52.00	56.00
Boerhavia diffusa (Nyctaginaceae)	Aqueous	Control	23.30	49.50	80.00	90.00	*	*	*
		1:1	19.50	48.00	84.00	90.00	*	*	*
		1:2	20.80	49.80	83.50	90.00	*	*	*
		1:5	24.80	51.50	76.50	90.00	*	*	*
	Methanol	Control	24.00	57.00	90.00	90.00	*	*	*
		1:1	18.30	45.30	77.30	90.00	*	*	*
		1:2	20.80	46.30	87.00	90.00	*	*	*
		1:5	20.50	52.80	90.00	90.00	*	*	*
Oxalis corniculata (Oxalidaceae;	Aqueous	Control	18.25	38.80	57.50	78.00	*	*	*
		1:1	13.50	36.80	66.00	90.00	*	*	*
		1:2	20.25	43.80	72.80	90.00	*	*	*
		1:5	24.25	47.50	75.80	90.00		*	*
	Methanol	Control	18.25	38.80	57.50	78.00	90.00	90.00	*
		1:1	12.30	32.20	45.25	57.15	70.00	90.00	*
		1:2	13.50	36.80	46.00	60.00	73.25	90.00	*
		1:5	16.50	37.25	48.05	64.80	79.00	90.00	*
Sida acuta (Malvaceae)	Aqueous	Control	19.00	47.50	83.00	90.00	*	*	*
		1:1	17.30	42.50	66.50	83.00	*	*	*
		1:2	18.30	45.30	76.00	89.00	*	*	*
		1:5	19.80	47.80	80.50	90.00	*	*	*
	Methanol	Control	14.50	35.00	58.30	87.50	*	*	*
		1:1	-	21.50	45.80	64.50	*	*	*
		1:2	7.80	26.30	52.00	75.30	*	*	*
		1:5	12.00	35.80	68.50	90.00	*	*	*

- No growth
*** Reading discontinued.**

Table 6

Vegetative growth of Helminthosporium sp. at 28°C on agar amended with varying dilutions of extracts of indicated plants.

Plant species	Type of extract	(v/v) !	Mean Diameter of culture (mm) after (days) 2 3 4 5 6 7
Alternanthera repens (Amaranthaceae)	Aqueous	Control	12.30 20.00 27.80 34.3043.30 57.50 59.00
		1:1	1 1.80 18.00 29.30 38.50 48.80 57.3063.25
		1:2	1 1.80 20.80 30.30 39.00 50.0057.80 64.00
		1:5	15.50 22.30 33.50 44.0055.80 67.5076.00
	Methanol	Control	8.00 14.75 18.5024.75 32.0042.25 52.00
		1:1	9.00 12.50 15.75 16.00 17.50 17.50 17.50
		1:2	9.50 12.33 12.75 13.00 13.00 13.00 13.00
		1:5	10.00 10.50 11.00 11.00 12.00 12.00 12.00
Boerhavia diffusa (Ny ctaginaceae)	Aqueous	Control	8.00 16.50 25.3033.80 45.00 52.30 59.30
		1:1	11.00 24.00 36.50 48.80 61.5073.80 82.00
		1:2	10.80 23.00 35.00 47.80 6 1.5072.00 82.50
		1:5	1 1.30 24.30 36.00 49.0063.00 73.50 81.50
	Methanol	Control	13.30 22.50 30.30 32.50 39.0057.30 64.50
		1:1	13.8024.50 34.00 37.0047.50 65.00 73.30
		1:2	13.80 23.80 36.00 37.0045.50 64.80 70.50
		1:5	13.80 25.30 35.00 35.5045.5064.50 71.00
Oxalis corniculata (Oxalidaceae)	Aqueous	Control	10.50 16.50 25.0031.00 38.5046.00 56.00
		1:1	13.50 2 1.00 28.00 42.50 44.7547.00 49.50
		1:2	12.30 19.30 28.50 36.30 43.6051.00 63.50
		1:5	10.50 18.30 26.50 35.0042.00 48.50 56.30
	Methanol	Control	9.00 17.00 34.50 27.50 42.5052.00 60.00
		1:1	7.00 16.50 34.0028.80 42.00 51.0559.00
		1:2	9.50 18.25 40.25 32.95 44.0058.00 68.00
		1:5	10.00 2 1.00 42.00 34.00 52.00 64.2572.00
Sida acuta (Malvaceae)	Aqueous	Control	10.75 21.25 31.00 42.25 53.2563.75 74.25
		1:1	10.50 19.00 30.00 39.0047.50 47.25 68.00
		1:2	8.00 18.25 27.0038.0048.00 59.00 71.00
		1:5	9.00 18.75 30.00 42.00 5 1.7562.50 68.50
	Methanol	Control	9.00 18.25 25.50 34.0042.00 44.00 57.00
		1:1	10.00 15.00 23.00 26.75 29.7537.25 5 1.50
		1:2	10.25 17.75 24.50 30.25 3 1.2536.25 52.25
		1:5	10.75 19.00 25.75 31.2533.2539.50 52.25

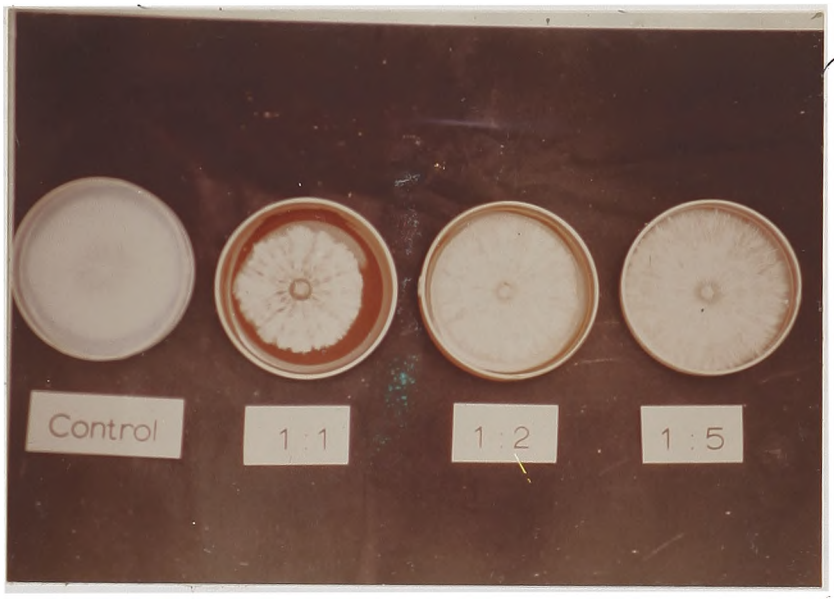


Plate 4. Vegetative growth of S. rolfsii at 28°C for 4 days on agar medium amended with indicated concentrations of the aqueous dry leaf extract of O. corniculata.
(Note the depression of growth at 1:1v/v dilution of the extract).

F. SCLEROTIA PRODUCTION BY S.ROLFSII ON AGAR AMENDED WITH AQUEOUS AND METHANOL LEAF EXTRACTS OF PLANTS IN THE FAMILIES AMARANTHACEAE. MALVACEAE.NYCTAGINACEAE AND OXALIDACEAE.

Both the aqueous and methanol leaf extract of the plants *Sida acuta* (Malvaceae), *Boerhavia diffusa* (Nyctaginaceae), *Oxalis corniculata* (Oxalidaceae) and *Alternanthera repens* (Amaranthaceae), variably depressed the number and size of sclerotia formed on agar medium. Generally, the aqueous extract of the plant yielded more toxic compounds which depressed sclerotia production (Table 7 and Fig.8) except in the case of *A. repens* where the inhibitory principle obtained in the methanol extract was more effective than what existed in the aqueous extract.The effectiveness of the 1:lv/v dilution of the aqueous extract can be ranked as following in descending order:

S. acuta (83.78%) > *B. diffusa* (56.77%) > *0. corniculata* (40.53%) > *A.repens* (36.16%) (% depression in all cases). Further dilution of the extract gradually decreased the inhibitory effect but did not alter the trend obtained above. (Table 7,Fig 8).

The fungus behaved differently in the medium amended with methanol extract of the plant species.The effectiveness of the methanol extract in depressing the sclerotia production can be ranked as follows (in descending order):

A.repens (94.62%) > *S.acuta* (59.30%) > *B.diffusa* (42.67%) > *O. corniculata* (11.22%) (% depression in all cases). Again, dilution of the extract gradually decreased the inhibitory effect but not the trend obtained. (Appendices 25a, 26a, 27a and 28a) summarises analysis of variance of data presented in Table 5 for the aqueous and methanol extracts respectively. Multiple range analysis of the data for aqueous and methanol extracts (Appendices 2 5b, 26b, 27b and 28b) confirm the trends obtained. The depression in sclerotia production was attended by a corresponding decrease in size of the sclerotia although the trend was not as straight-forward as for the decline in number.

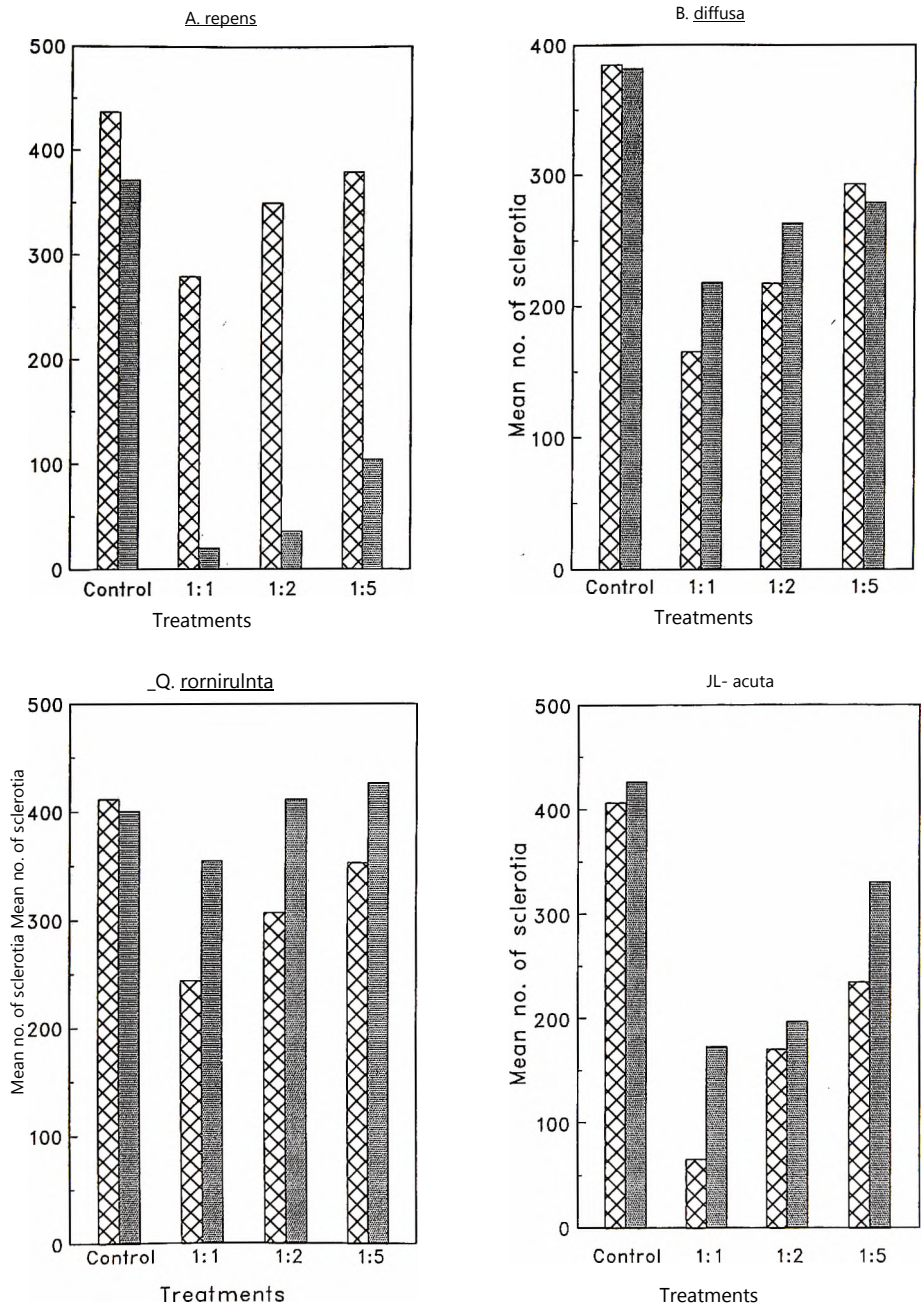
Table 7

Sclerotia production by Sclerotium rolfsii at 28°C on agar medium amended with varying dilutions of extracts of indicated plants.

Plant species	Nature of extract	Dilution of extract (v/v)	No. of sclerotia Mean + S.E		% depression Dia.	of sclerotia (mm) Mean ± S.E	
Alternanthera repens (Amaranthaceae)	Aqueous	1:1	279	3.52	36.16	68.80	2.18
		1:2	350	0.41	19.90	66.97	2.28
		1:5	379	5.89	13.27	78.08	0.60
		Control	437	1.22	-	82.72	1.09
	Methanol	1:1	20	2.04	94.62	58.29	2.74
		1:2	36	3.52	90.32	75.50	2.63
		1:5	104	1.68	72.04	78.79	1.09
		Control	372	2.48	-	87.92	2.25
Boerhavia diffusa (Nyctaginaceae)	Aqueous	1:1	166	3.49	56.77	62.01	2.28
		1:2	218	6.38	44.53	61.84	1.83
		1:5	298	2.68	22.96	64.68	1.13
		Control	384	3.20	-	87.21	1.70
	Methanol	1:1	219	2.29	42.67	71.26	1.92
		1:2	264	4.02	30.89	77.49	1.85
		1:5	280	4.76	26.70	81.66	1.29
		Control	382	2.58	-	87.21	1.70'
Oxalis corniculata (Oxalidaceae)	» Aqueous	1:1	245	4.56	40.53	55.08	2.28
		1:2	308	5.95	25.24	69.05	1.35
		1:5	354	6.65	14.08	69.55	2.51
		Control	412	4.42	-	75.93	1.06
	Methanol	1:1	356	4.08	12.53	67.13	1.01
		1:2	412	11.46	NDO	74.30	1.04
		1:5	427	2.97	NDO	75.78	0.61
		Control	407	1.04	-	75.93	1.06
Sida acuta (Malvaceae)	Aqueous	1:1	66	2.58	83.78	79.45	1.96
		1:2-	171	2.48	57.99	84.33	1.52
		1:5	236	2.94	42.01	82.61	1.57
		Control	407	6.12	-	71.98	1.70
	Methanol	1:1	174	. 2.61	59.25	71.99	1.84
		1:2	198	4.53	53.63	76.24	1.84
		1:5	332	4.97	22.25	81.92	1.56
		Control	427	2.61	-	75.77	1.44

NDO —No depression occured.

Fig 8



Aqueous I Methanol

Sclerotia production by S. rolfsii on agar amended with indicated dilutions of plant extracts.

G. VEGETATIVE GROWTH OF THE MYCELIUM OF *S. ROLFSII* AND *HELMINTHOSPORIUM* SP. ON AGAR MEDIUM AMENDED WITH EXTRACTS OF PLANTS BELONGING TO THE FAMILIES MELIACEAE AND RUTACEAE.

Both aqueous and methanol extracts of *A. indica, C. anisata* and *Z. xanthoxyloides* exerted marginal inhibition effect on the vegetative growth of *S. rolfsii* and *Helminthosporium* at least at the highest dilution applied in liquid medium. On agar, the results were variable (Tables 8 and 9). The aqueous extract of C. *anisata* showed no significant difference on the vegetative growth of the two test fungi at (P =s 0.05). *Z. xanthoxyloides* and *A. indica* water extracts showed significant inhibitory effect (P s 05) on the vegetative growth of *S. rolfsii* and *Helminthosporium* sp. with the water extract of *Z. xanthoxyloides* suppressing the growth of *S. rolfsii* to a greater extent than *Helminthosporium* sp. *Helminthosporium* sp. was significantly (P s 0.05) suppressed by *A. indica* extracts (Appendices 29-33). Further dilution of the extract removed the inhibitory effect in all instances (Tables 8 and 9).

Table 8

Vegetative growth of Sclerotium rolfsii at 28°C on agar amended with varying dilutions of extracts of indicated plants.

Plant species	Type of extract	Dilution ratio (v/v)	Mean Diameter of culture (mm) after (days) 1	2	3	4	5	6
Azadirachta indica (Meliaceae)	Aqueous	Control	18.25	38.80	57.50	78.00	*	*
		1:1	13.50	36.80	66.00	90.00	*	*
		1:2	20.25	43.80	72.80	90.00	*	*
		1:5	24.25	47.50	75.80	90.00	*	*
	Methanol	Control	18.25	38.80	57.50	78.00	90.00	*
		1:1	12.30	32.20	45.25	57.15	70.00	*
		1:2	13.50	36.80	46.00	60.00	73.25	*
		1:5	16.00	37.25	48.05	64.00	79.00	*
Clausena anisata (Rutaceae)	Aqueous	Control	-	10.00	31.00	62.00	90.00	90.00
		1:1	-		23.00	45.00	71.50	78.00
		1:2	-	10.00	32.00	53.5	77.00	88.00
		1:5		9.5	30.30	56.5	83.50	90.00
	Methanol	Control	11.00	31.25	51.00	81.00	90.00	90.00
		1:1	_	8.50	8.50	11.50	13.50	15.50
		1:2		8.75	17.50	19.25	23.00	27.25
		1:5		11.75	23.25	30.75	35.50	40.25
Zanthoxylum xanthoxyloides (Rutaceae)	Aqueous	Control	—	12.50	35.50	58.00	87.50	90.00
		1:1		9.00	26.50	53.00	81.80	88.80
		1:2	-	11.80	30.50	53.80	81.00	88.00
		1:5	-	11.00	33.30	62.80	90.00	90.00
	Methanol	Control	10.00	40.00	74.80	90.00	*	*
		1:1	12.00	31.30	51.00	75.00	*	
		1:2	11.30	32.80	56.00	83.50	*	*
		1:5	14.00	40.00	69.30	90.00	*	*

- No growth
* Reading discontinued

Table 9

Vegetative growth of Helminthosporium sp. at 28°C on agar medium amended with varying dilutions of extracts of indicated plants.

Plant species	Type of extract	Dilution ratio (v/v)	Mean Diameter of culture (mm) after (days) 1	2	3	4	5	6	7
Azadirachta indica (Meliaceae)	Aqueous	Control	9.00	19.75	28.80	36.50	41.75	52.50	66.00
		1:1	10.00	16.25	22.15	28.00	33.50	40.60	48.00
		1:2	9.50	17.25	22.00	27.65	32.40	37.68	44.00
		1:5	9.00	17.00	26.00	34.00	42.50	50.50	64.00
	Methanol	Control	-	14.50	27.60	33.00	39.75	45.61	56.75
		1:1		10.00	18.00	26.70	33.30	44.00	54.00
		1:2	-	10.50	22.15	30.50	4 1.25	47.75	57–25
		1:5		13.00	26.90	35.90	44.45	51.40	60.55
Clausena anisata (Rutaceae)	Aqueous	Control	9.80	19.00	28.30	39.30	52.00	59.50	70.00
		1:1	1 1.00	17.50	24.30	31.80	36.50	43.80	54.00
		1:2	12.30	18.00	25.30	33.00	39.50	47.50	56.00
		1:5	11.80	20.30	29.50	38.30	46.00	5 1.50	62.00
	Methanol	Control	-	14.75	18.50	24.75	32.00	42.25	52.00
		1:1	-		8.50	11.00	13.00	15.00	18.50
		1:2	-	9.75	12.00	13.25	15.00	16.75	21.00
		1:5	—	13.50	18.25	24.75	27.50	30.00	40.25
Zanthoxylum xanthoxyloides (Rutaceae)	Aqueous	Control	10.80	18.50	28.30	37.00	47.30	55.00	73.50
		1:1	10.80	19.80	28.30	38.00	46.30	53.50	66.30
		1:2	1 1.00	2 1.30	32.80	44.50	53.50	62.00	70.80
		1:5	9.50	19.50	31.00	42.50	53.50	63.50	67.50
	Methanol	Control	-	14.75	18.50	24.75	32.00	42.25	52.00
		1:1	8.00	13.00	14.25	16.00	18.75	19.00	19.00
		1:2	10.00	13.75	14.25	14.75	18.00	18.25	18.75
		1:5	9.00	13.50	13.75	15.00	18.25	18.25	18.25

- No growth

H. VEGETATIVE GROWTH OF *S. ROLFSII* AND *HELMINTHOSPORIUM* SP. IN BROTH MEDIUM AMENDED WITH EXTRACTS OF PLANTS IN THE FAMILIES MELIACEAE AND RUTACEAE.

Aqueous extract.

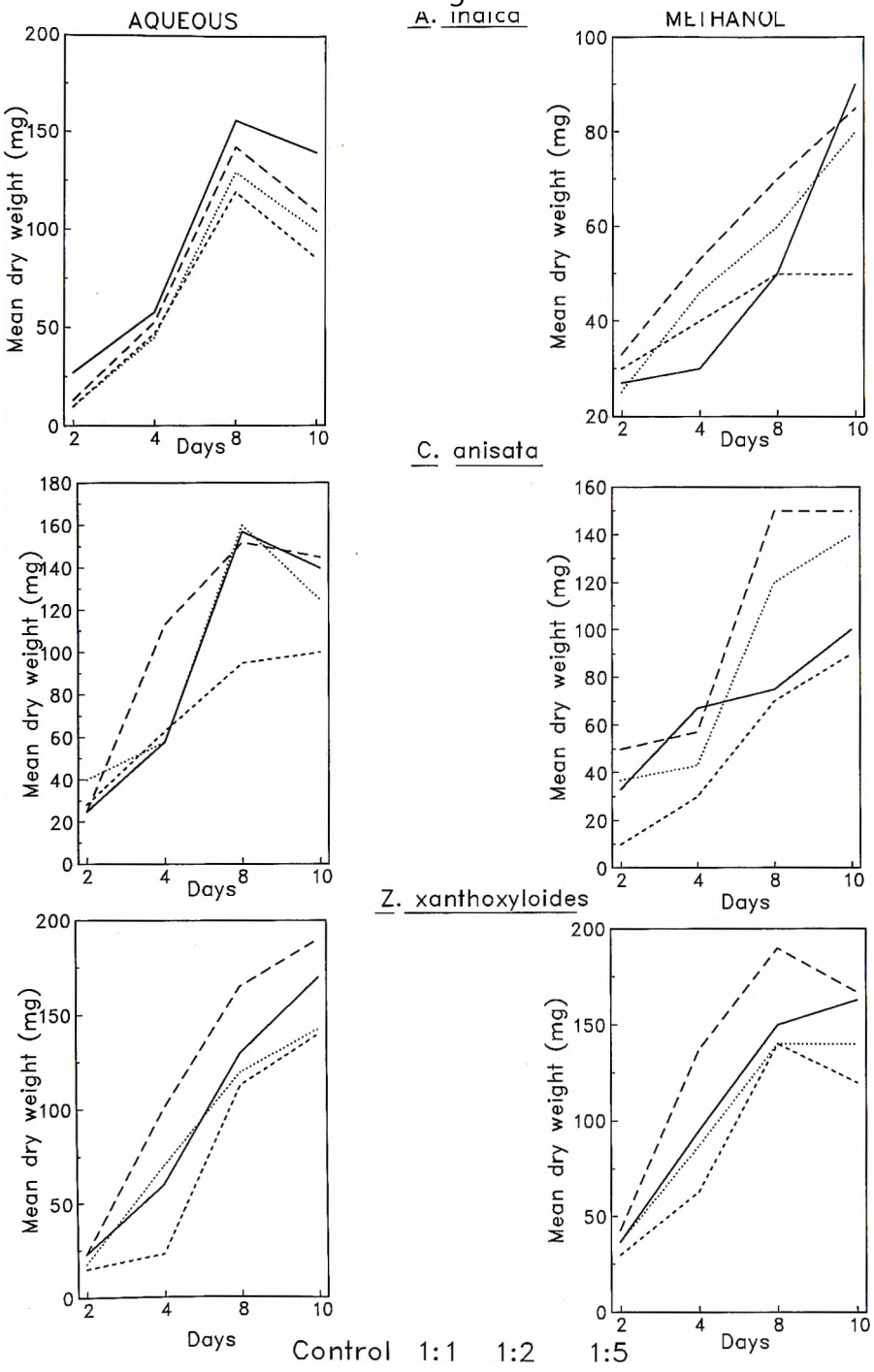
Results obtained are summarised in Figs. 9 and 10 and Appendices 34a-c. The inhibitory effect of vegetative growth of *S. rolfsii* and *Helminthosporium* sp. by the aqueous extract of *Clausena anisata* (Rutaceae), *Zanthoxylum xanthoxyloides* (Rutaceae) and *Azardirachta indica* (Meliaceae) could be described as marginal. Inhibition was greatest at l:lv/v dilution but this effect was removed with increasing dilution (up to l:5v/v dilution). Statistical test of data (Appendices 34a-c) showed that the inhibitory effect of the aqueous extract of the test plant was severer on *Helminthosporium* sp. than on *S. rolfsii* at the highest (l:lv/v dilution) concentration of the extract.

Methanol extract.

Again the methanol extract of the plant extracts showed marginally inhibitory effect on vegetative growth of *S. rolfsii* and *Helminthosporium* sp. at least at the highest dilution (l:lv/v dilution) used. The inhibitory effect was gradually removed with increasing dilution of all the alcohol extracts. Multiple range analysis showed that the

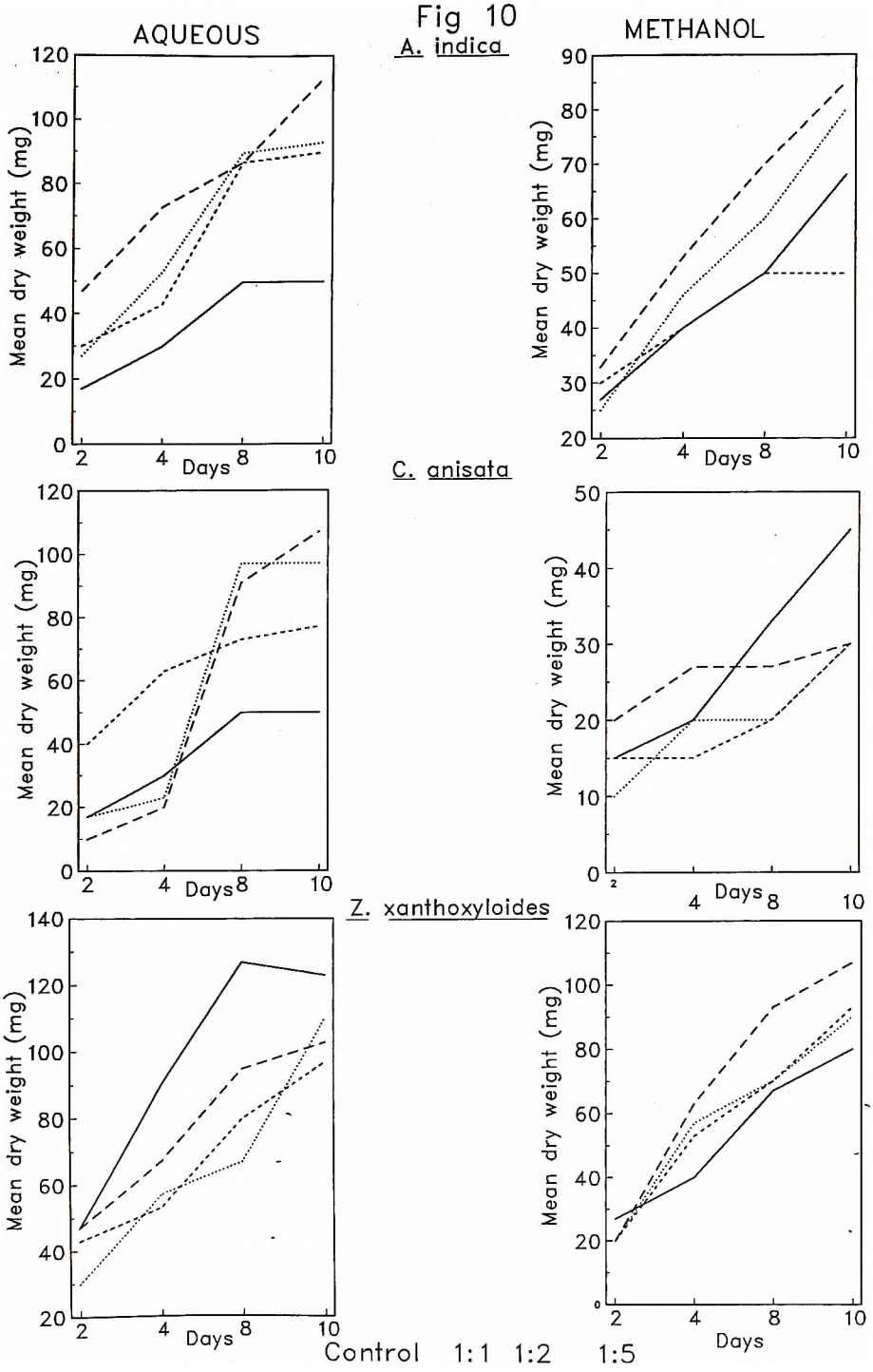
inhibitory effect of the methanol extracts, although minimal was severer on *Helminthosporium* sp. than on *S. rolfsii.*

Fig 9



Vegetative growth of S. rolfsii in liquid medium amended with the aqueous and methanol extracts of A. indica, C. anisata and Z. xanthoxyloides.

Fig 10



Vegetative growth of Helminthosporium sp.in liquid medium amended with aqueous and methanol extracts of _A. indica, C. a-nisata and 7. xnnthoxyloides.

I . PRODUCTION OF SCT.EROTIA BY *S. ROLFSII* ON AGAR AMENDED WITH EXTRACTS OF PLANTS IN THE FAMILIES MELIACEAE AND RUTACEAE.

Aqueous extract.

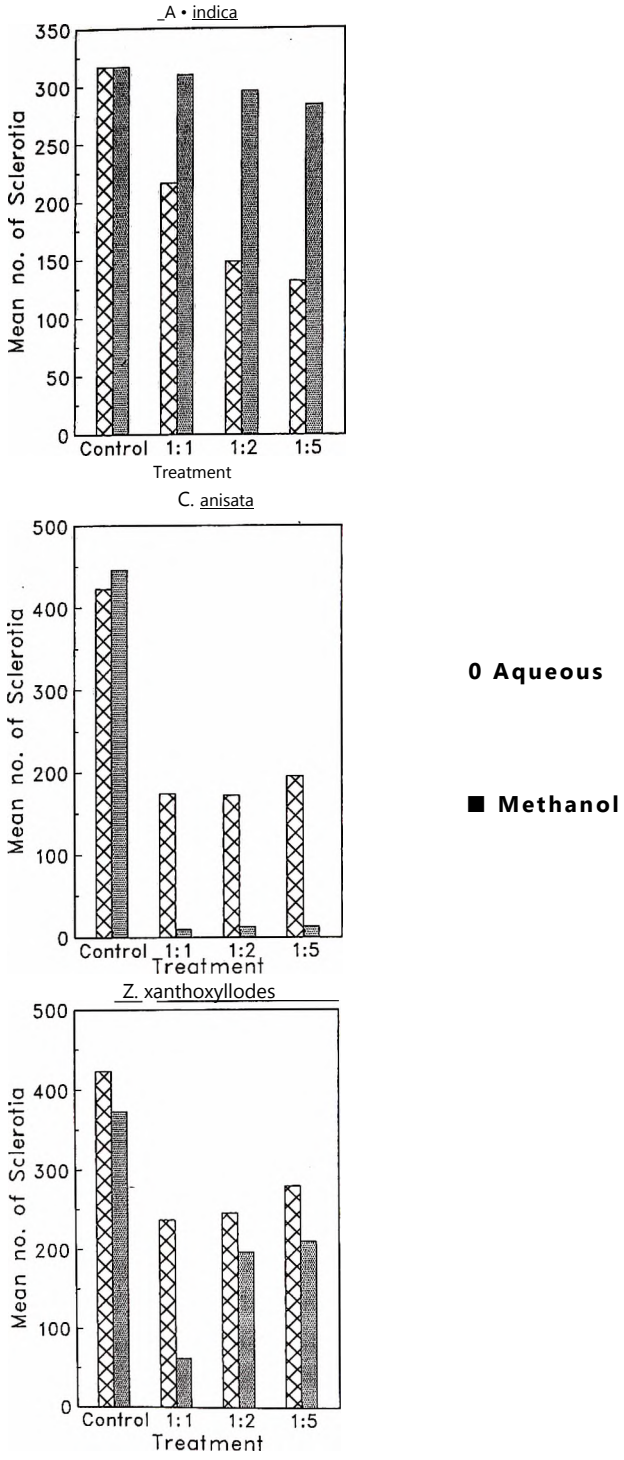
Results obtained are summarised in (Fig. 11, Table 10 and Appendices 35-38). There was a significant difference (P a 0.05) between the number of sclerotia formed on agar medium amended with aqueous extract of *A.indica, C.anisata* and *Z.xanthoxyloides* and the control unamended basal medium agar.(Fig. 11, Appendices 35-3 8). The number of sclerotia formed was in the descending order : *C. anisata* < *Z. xanthoxyloides* < *A.indica.* There was however no significant difference (P s 0.05) between the dimensions of the sclerotia formed in the amended media and the control (Appendices 35a and 35b).

Ethanol extract.

The ethanol extracts of the test plants also depressed sclerotia production (Fig 11, Appendices 38a and 38b). Although there was a significant difference between the number of sclerotia formed in the control and in the agar medium amended with ethanol extract of *A.indica, C.anisata* and *Z.xanthoxyloides,* the dimensions of the sclerotia formed in *Z. xanthoxyloides* and *A.indica* did not differ significantly at P s 0.05 (Fig. 11, Appendices 36a and

36b). The depressive effect of extract on sclerotia number and size was not removed with increasing dilution of the extracts (Fig. 11).

Fig 1 1



Sclerotia production by S. rolfsii on agar amended with indicated plant extract.

Table 10

Sclerotia production on agar amended with varying dilutions of extracts of indicated plants.

Plant species extract	Nature of	Dilution of extract (v/v)	No. of sclerotia Mean ± S.E		% depression	Dia. of sclerotia (mr Mean- ± S.E	
Azadirachta indica (Meliaceae)	Aqueous	. 1:1	217	3.51	31.53	69.69	1-26
		1:2	149	4.20	53.31	74.10	1.11
		1:5	132	3.37	58.36	87.50	1.88
		Control	317	9.45	-	78.31	1.18
	Methanol	1:1	310	17.17	2.21	73.31	2.21
		1:2	296	5.84	5.99	75.69	2.1 1
		1:5	284	8.99	10.41	73.56	0.80
		Control	317	9.47	-	78.31	1.18
Clausena anisata (Rutaceae)	Aqueous	1:1	175	1.04	58.53	63.83	1.12
		1:2	173	1.04	59.00	69.17	1.57
		1:5	196	1.68	53.55	76.04	2.29
		Control	422	3.01	-	94.62	4.04
	Methanol	1:1	10	0.82	97.76	101.13	4.26
		1:2	13	2.84	97.09	79.13	3.35
		1:5	14	0.87	96.87	69.88	2.99
		Control	447	2.97	-	94.63	4.04
Zanthoxylum xanthoxyloides (Rutaceae)	Aqueous	1:1	238	1.63	43.87	64.00	2.14
		1:2	247	11.27	41.75	68.81	2.43
		1:5	281	2.04	33.73	75.88	0.389
		Control	424	1 1.78	-	80.96	2.02
	Methanol	1:1	63	3.29	83.16	70.64	1.73
		1:2	149	4.84	60.43	77.74	1.70
		1:5	209	7.84	44.39	73.09	1.85
		Control	374	4.40	-	80.96	2.02

J. VEGETATIVE GROWTH OF *S. ROLFSII* AND *HELMINTHOSPORIUM* SP. ON AGAR MEDIUM AMENDED WITH LEAF EXTRACTS OF PLANTS IN THE FAMILIES APOCYNACEAE AND RUBIACEAE.

Results obtained are presented in (Tables 11-14) and in Plate 5. Depression in growth of the test fungi {*S.rolfsii* and *Helminthosporium* sp.) in 1:lv/v dilution of both aqueous and methanol leaf extract of *C. roseus* and *M. inermis* can be described as marginal. Further dilutions of the extract completely removed the inhibitory effect and in some instances improved vegetative growth (Tables 11 and 12; Plate 5). Statistical analysis (Scheffe average) in Tables 13b,14a show that the meagre depression in growth was severer in most cases on *Helminthosporium* sp than for *S.rolfsii* except the methanol extract of *M. inermis* (Table 14b) .

Table 11

Vegetative growth of Sclerotium rolfsii at 28°C on agar medium amended varying dilutions of extract of indicated plants

Plant species Type	of extract	Dilution ratio (v/v)	Mean Diameter of culture (mm) after (days)					
			1	2	3	4	5	6
Catharanthus roseus (Apocynaceae)	Aqueous	Control	1	1.50	35.80	65.30	90.00 *	*
		1:1		12.30	38.80	68.00	90.00 *	*
		1:2		12.30	42.00	70.80	90.00 *	*
		1:5		14.30	43.00	73.00	90.00 *	*
	Methanol	Control		14.30	46.00	80.80	90.00 *	*
		1:1		12.30	31.50	55.00	76.30	90.00 *
		1:2		13.50	38.00	64.80	87.80	90.00 *
		1:5		16.30	44.80	75.80	90.00 *	*
Mitragyna inermis (Rubiaceae;	Aqueous	Control		18.25	38.80	57.50	78.00	90.00 *
		1:1		28.00	46.50	76.50	90.00 *	*
		1:2		28.00	45.80	76.30	90.00 *	*
		1:5		30.25	49.00	79.30	90.00 *	*
	Methanol	Control	1	1.70	17.50	25.00	56.00	79.00 90.00
		1:1		-	19.50	26.00	50.00	65.75 82.50
		1:2		-	18.25	25.00	49.25	65.50 82.50
		1:5		-	17.75	21.00	50.75	72.80 90.00

— No growth
*** Reading discontinued.**

Table 12

Vegetative growth of Helminthosporium sp. at 28°C on agar medium amended with varying dilutions of extracts of indicated plants.

Plant species	Type of extract	Dilution ratio (v/v)	Mean Diameter of culture (mm) after (days)						
			1	2	3	4	5	6	7
Catharanthus roseus (Apocynaceae)	Aqueous	Control	10.30	18.50	26.30	32.30	41.00	46.50	55.00
		1:1	10.50	23.80	31.00	39.30	48.00	57.80	64.50
		1:2	11.00	22.50	32.50	41.00	51.00	59.50	70.50
		1:5	12.30	22.50	33.80	43.80	54.50	64.50	72.50
	Methanol	Control	10.80	20.00	28.30	35.50	43.30	50.80	60.00
		1:1	11.80	21.80	31.00	38.80	46.80	53.00	60.30
		1:2	11.50	20.80	31.50	39.50	50.80	59.30	64.80
		1:5	12.30	22.50	33.50	42.80	51.80	60.00	67.30
Mitraayna inermis (Rubiaceae)	Aqueous	Control	9.00	19.75	28.80	36.50	41.50	52.50	66.00
		1:1	9.00	16.25	26.25	27.00	34.00	39.00	48.00
		1:2	10.00	16.25	26.50	28.00	34.25	38.50	47.50
		1:5	8.75	15.75	24.75	28.25	32.25	37.50	42.00
	Methanol	Control	10.75	21.25	31.00	42.25	53.25	63.75	74.25
		1:1	4.00	13.75	19.50	26.91	34.50	40.50	48.85
		1:2	9.25	17.00	23.75	31.30	39.50	46.50	55.00
		1:5	11.50	21.00	28.75	38.70	49.00	59.50	70.71

Table 13a

Multiple range analysis of aqueous extract of C. roseus on the vegetative growth of indicated fungi on solid medium. (Note differences between Scheff Averages for both fungi.)

Fungus	Count	Average	Homogeneous Group
Helminthosporium sp.	16	65.5	A
S. rolfsii	16	84.5	B

Table 13b

Multiple range analysis of aqueous extract of M.inermis on the vegetative growth of indicated fungi on solid medium. (Note differences between Scheff Averages for both fungi.)

Fungus	Count	Average	Homogeneous Group
Helminthosporium sp.	16	50.875	A
S. rolfsii	16	87.000	B

Figures with different letters are significantly different.

Table 14a

Multiple range analysis of methanol extract of C. roseus on the vegetative growth of indicated fungi on solid medium. (Note differences between Scheff Average for both fungi.)

Fungfts	Count	Average	Hombgeneous Group	
Helminthosporium sp.	16 >	63.0625	**A**	
S. rolfsii	16	86.0000		**B**

Table 14b

Multiple range analysis of methanol extract of M. inermis on the vegetative growth of indicated fungi on solid medium. (Note differences between Scheff Average for both fungi)

Fungus	Count	Average	Homogeneous Group	
S. rolfsii	16	51.5000	**A**	
Helminthosporium sp.	16	62.1875		**B**

Figures with different letters are significantly different.

(>S

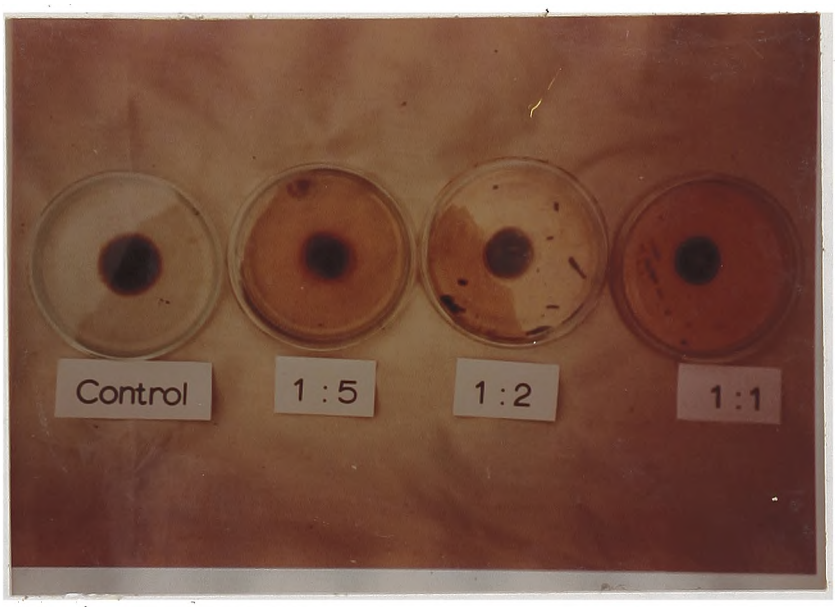


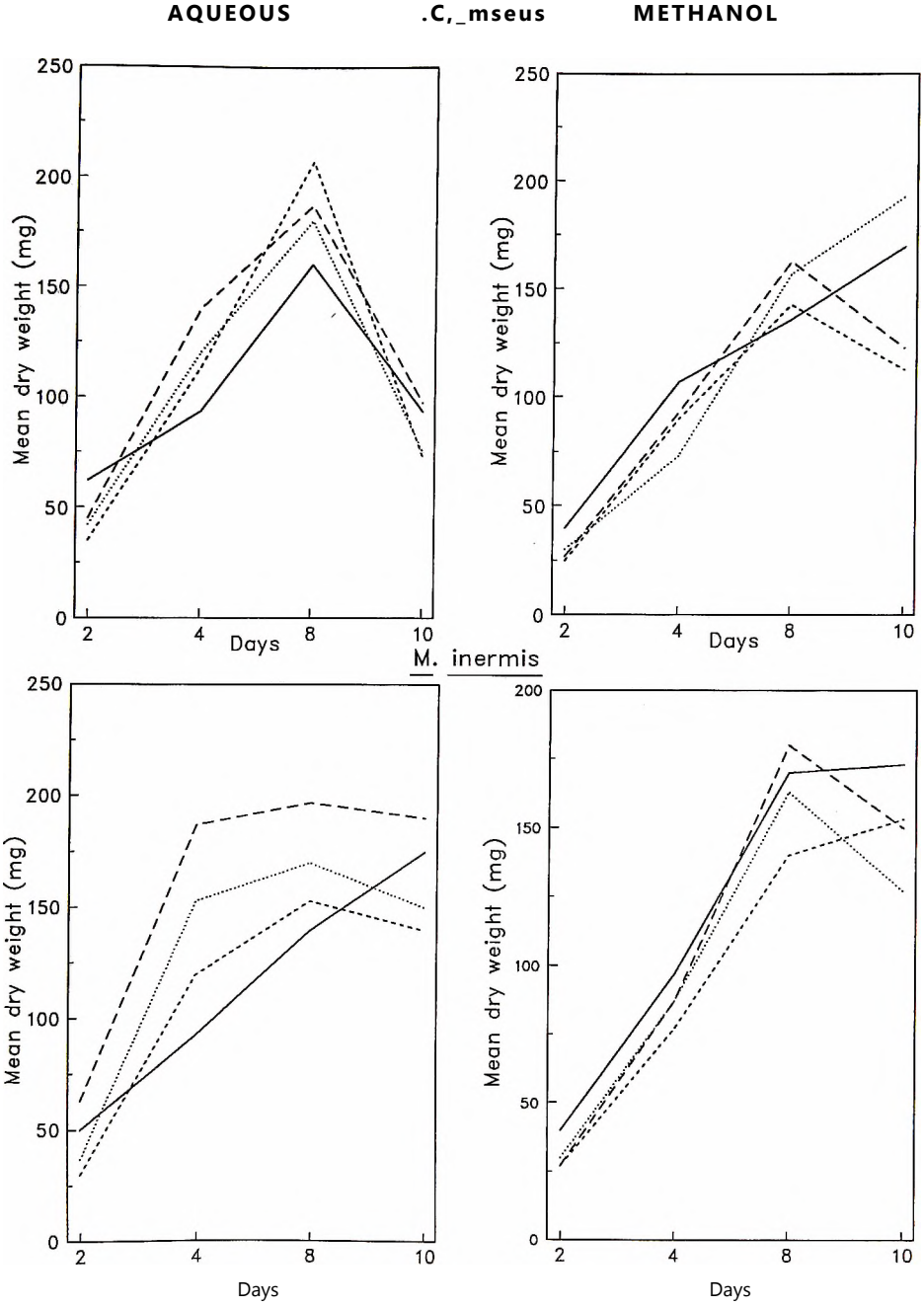
Plate 5. Radial growth of Helminthosporium sp. at 28°C for 5 days amended with aqueous dry leaf extract of M. inermis. (Note the meagre depression of radial growth by all concentrations of the extract).

K. VEGETATIVE GROWTH OF *S. ROLFSII* AND *HELMINTHOSPORIUM* SP IN BROTH MEDIUM AMENDED WITH LEAF EXTRACTS OF PLANTS IN THE FAMILIES APOCYNACEAE AND RUBIACEAE.

Aqueous and methanol extracts.

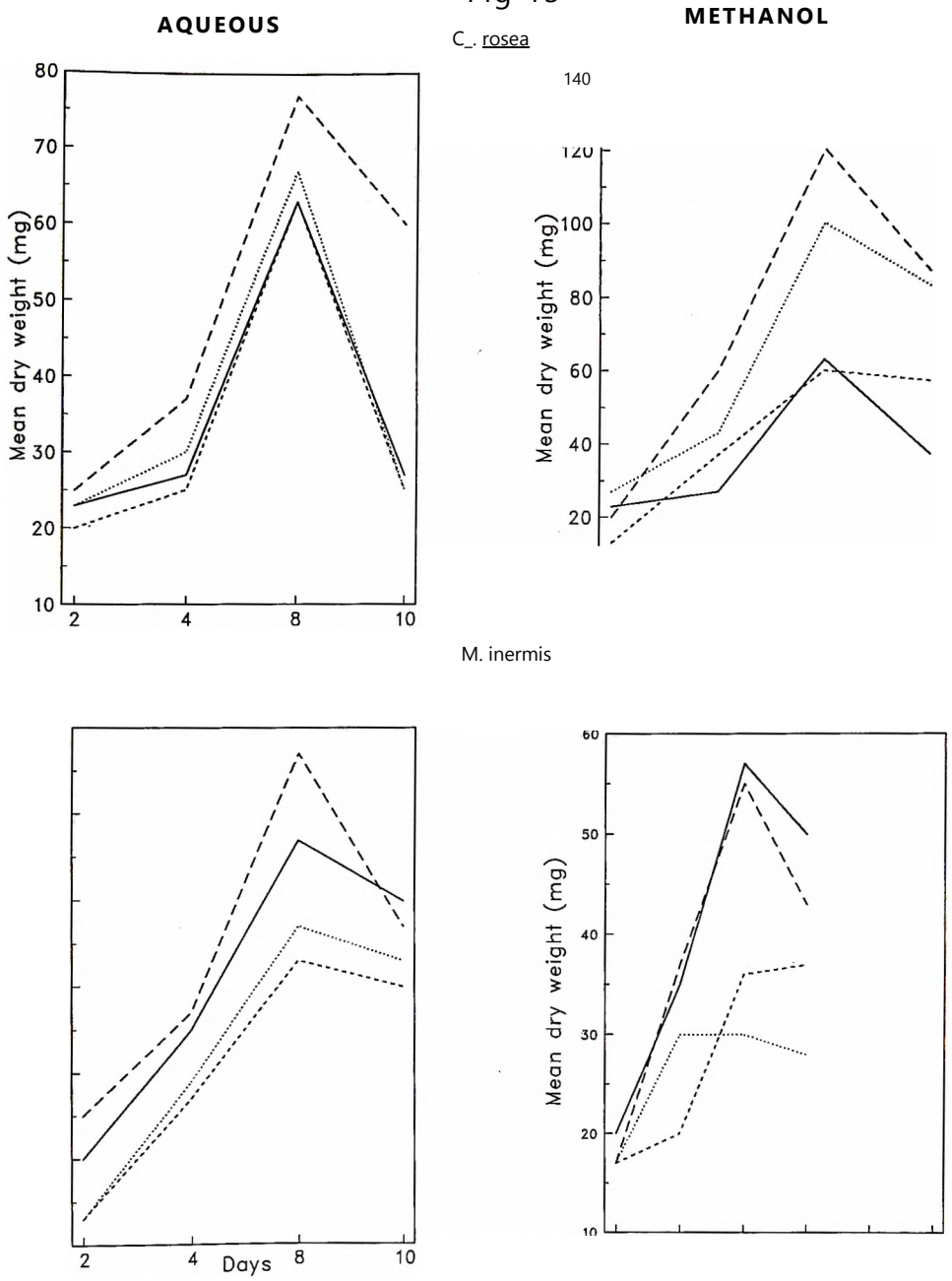
Results obtained on solid medium (Chapter J) did not differ significantly from what existed in liquid medium. The extracts of C. *roseus* and *M. inermis* marginally depressed vegetative growth of both *S. rolfsii* and *Helminthosporium* sp.(Figs 12 and 13). The depression of growth was observed during the first two days under the influence of the extracts and thereafter declined (Figs 12 and 13). Dilution of the extracts gradually removed the inhibitory effect to such an extent that in some instances growth in the l:5v/v dilution was better than in the control. Generally, the weak inhibitory effect of the extracts observed was severer on *Helminthosporium* sp than on *S. rolfsii* (Tables 15a, 15b, 16a and 16b).

Fig 12



Vegetative growth of S. rolfsii in liquid medium amended with aqueous and methanol extracts of _C.roseus and M. inermis.

Fig 13



Vegetative growth of Helminthosporium sp. in liquid medium amended with aqueous and methanol extracts of indicated plants.

Table 15a

Multiple range analysis of methanol extract of C. roseus on the vegetative growth of indicated fungi in liquid medium. (Note differences between Scheff Averages for both fungi.)

Fungus	Count	Average	Homogeneous Group
Helminthosporium sp.	16	85.8125	**A**
S. rolfsii	16	149.9375	**B**

Table 15b

Multiple range analysis of methanol extract of M. inermis on the vegetative growth of indicated fungi in liquid medium. (Note differences between Scheff Averages for both fungi)

Fungus	Count	Average	Homogeneous Group
Helminthosporium sp.	16	42.0000	**A**
S. rolfsii	16	163.125	**B**

Figures with different letters are significantly different.

Table 16a

Multiple range analysis of aqueous extract of C. roseus on the vegetative growth of indicated fungi in liquid medium. (Note differences between Scheff Averages for both fungi.)

Fungus	Count	Average	Homogeneous Group	
Helminthosporium sp.	16	67.500	**A**	
S. rolfsii	16	183.375		**B**

Table 16b

Multiple range analysis of aqueous extract of M.inermis on the vegetative growth of indicated fungi in liquid medium. (Note differences between Scheff Averages for both fungi.)

Fungus	Count	Average	Homogeneous Group	
Helminthosporium sp.	16	51.25	**A**	
S. rolfsii	16	166.25		**B**

Figures wifh different letters are significantly different.

L. PRODUCTION OF SCLEROTIA BY *S. ROLFSII* IN MEDIUM AMENDED WITH LEAF EXTRACTS OF PLANTS IN THE FAMILIES APOCYNACEAE AND RUBIACF.R-F:

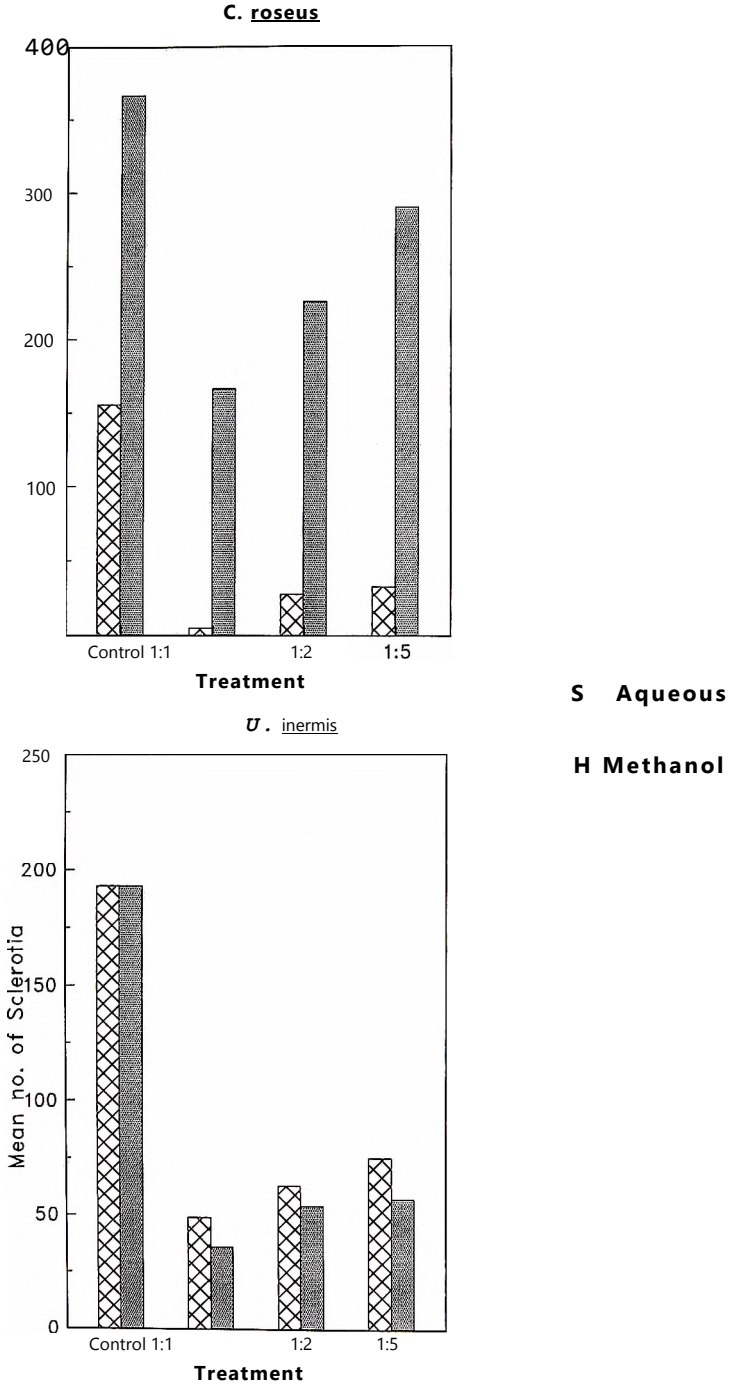
A summary of results obtained is as follows:
Both aqueous and methanol extracts of the leaves of the two plants, C. roseus and *M. inermis* at high concentrations depressed sclerotia formation (Table 17, Fig 14). The inhibitory effect was severer in extracts of *C. roseus* than *M. inermis* except in the case of methanol extract of C. *roseus* which permitted more sclerotia formation as compared to what existed in the methanol extract of *M. inermis.* There was significant (P 0.05) difference between the number of sclerotia produced in the control and the media amended with both the aqueous and the methanol extracts of C. *roseus* and *M. inermis* (Appendices 39 and 40). The aqueous extract of *C. roseus* was more depressive in its effect on sclerotia production by *S. rolfsii* than the equivalent methanol extract. The adverse was true for the extracts of *M. inermis.* Interestingly, the inhibitory effect was decreased by increasing dilution of the extracts but sclerotia formed never approximated what obtained in the control. Secondly depression in sclerotia formation was attended by increase in the size of the fewer numbers that were formed.

Table 17

Influence of the indicated dilutions of aqueous and methanol leaf extracts of C. roseus and M. inermis on sclerotia production on agar medium incubated at 28°C for 14 days.

Plant species	Nature of extract	Dilution of extract (v/v)	Sclerotia production Mean ± S.E		% depression	Dia. of sclerotia (um' Mean	± S.E
Catharanthus roseus (Apocynaceae)	Aqueous	1:1	5	1.22	96.79	68.26	2.27
		1:2	28	1.83	82.05	74.11	2.47
		1:5	33	1.29	78.88	75.59	1.34
		Control	156	4.80	-	75.44	0.83
	Methanol	1:1	167	5.21	54.49	77.12	0.73
		1:2	226	2.48	38.42	79.00	1.00
		1:5	290	6.66	20.98	83.38	1.88
		Control	367	6.36	-	83.92	3.24
Mitraqyna inermis (Rubiaceae)	Aqueous	1: 1	49	1.83	74.61	87.25	2.69
		1:2	63	1.47	67.35	72.70	1.00
		1:5	75	2.04	61.14	88.10	1.47
		Control	193	1.47	-	86.30	0.95
	Methanol	1:1	36	1.31	81.35	89.12	3.46
		1:2	54	1.83	72.02	103.46	1.40
		1:5	57	1.22	70.46	1 11.24	3.61
		Control	193	1.47	-	87.25	2.69

Fig 14



Sclerotia production by S. rolfsii on agar amended with indicated plant extracts.

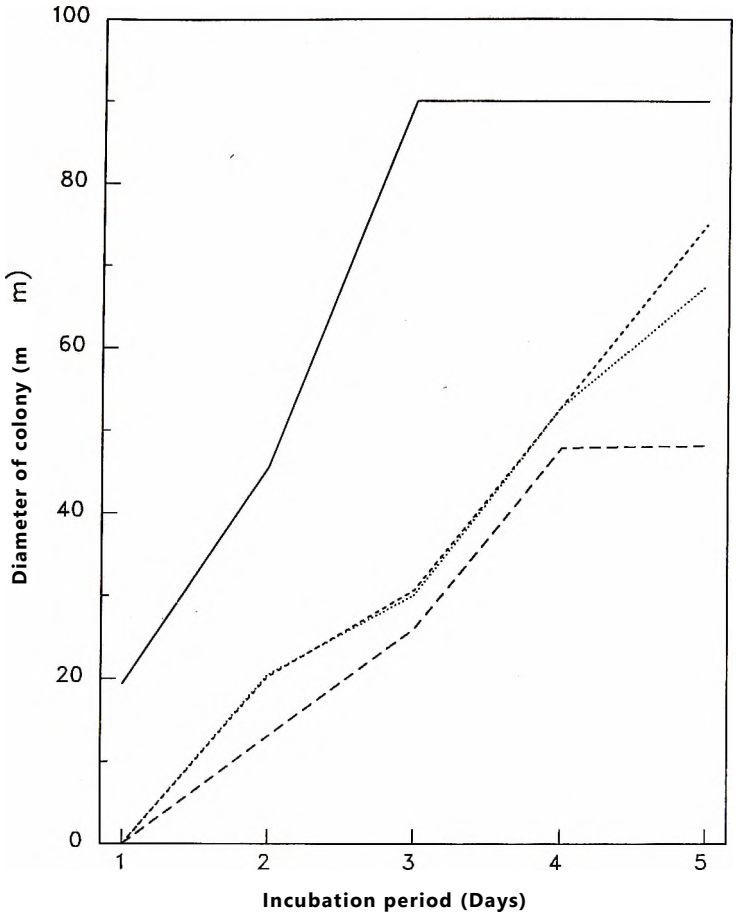
M. VEGETATIVE GROWTH OF *SCLEROTIUM ROLFSII* AND *HELMINTHOSPORIUM* SP. BURIED IN THE METHANOL EXTRACT OF *CASSIA ALATA* FOR VARYING PERIODS.

It required a minimum immersion period of 3 0 minutes (1/2h) in the leaf extract of *C. alata* to impair the vegetative growth of the mycelium of *S. rolfsii* and *Helminthosporium* sp.. The longer the period of immersion the severer the depression in the dry weight of harvested mycelium. (Figs 17 and 18). The methanol extract of C. *alata* depressed vegetative growth by 70-80 percent depending on the period of immersion. Statistical analysis of data showed that the difference observed are significant at (P s 0.05) (Appendices 41-47) .

On agar (solid medium) both vegetative growth and sclerotia production by *S. rolfsii* was completely prevented when the mycelium was buried in the methanol extract of *C. alata* for 48hrs prior to inoculating on extract-free medium(Fig 15 and Appendix 43b). In the remaining instances, the number of sclerotia formed was depressed by 17.24-40.2 percent. Vegetative growth of *Helminthosporium* sp. on agar was depressed by 20.49 percent when its mycelium was immersed in the methanol extract of C. *alata* for 48hrs prior to being transferred into extract free medium (Fig.16).

Fig. 15

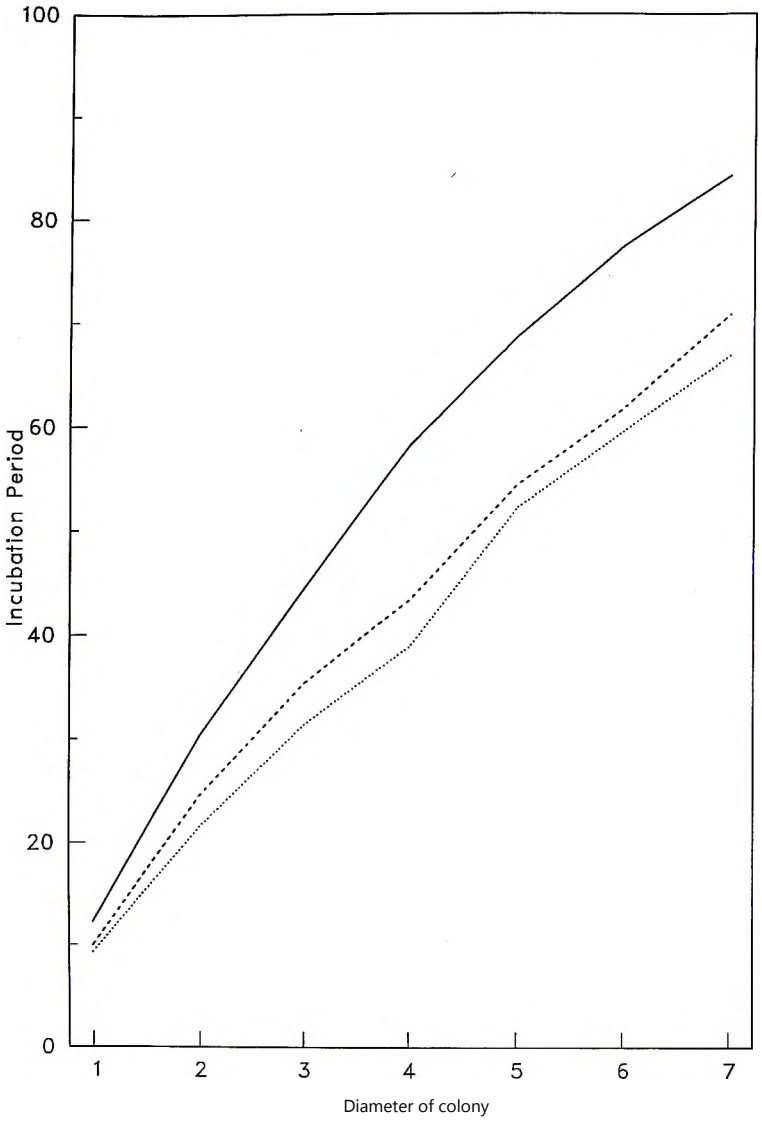
Radial growth of S. rolfsii buried in undiluted methanol extract of C. alata for the indicated periods before plating on solid medium at 28°C for 14 days.



Control 6 12 24. 48

Fig 16

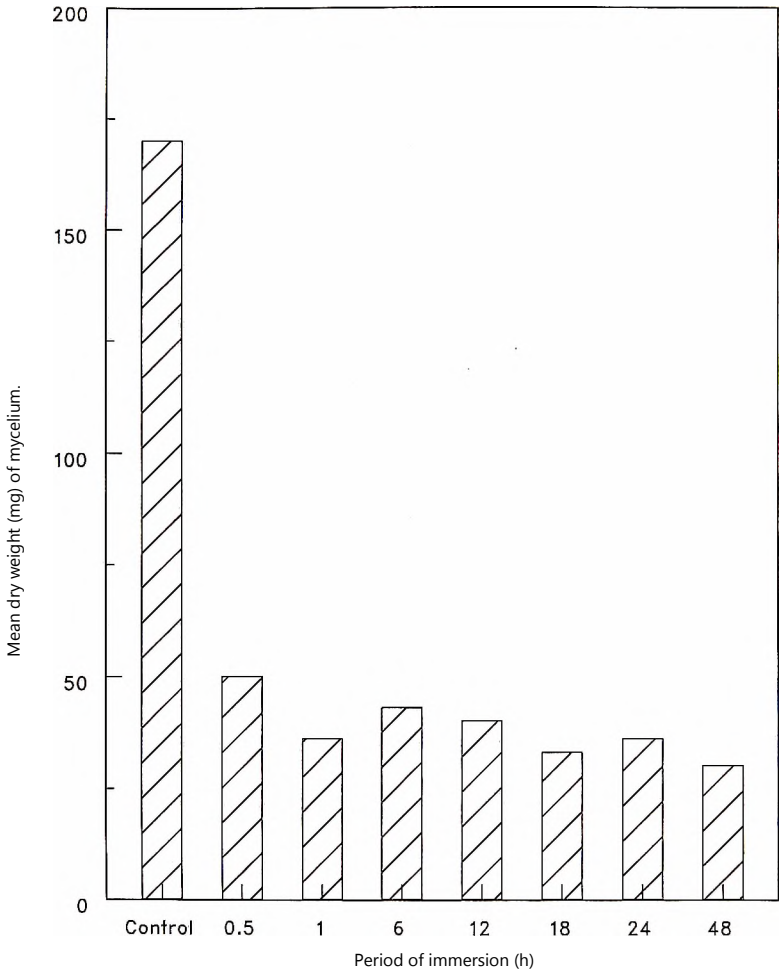
Radial growth of the mycelium of Helminthosporium sp. buried in undiluted methanol leaf extract of _C. alata for the indicated periods before plating on solid medium at 28 C for 7 days.



Control 24h 48h

Fig. 17

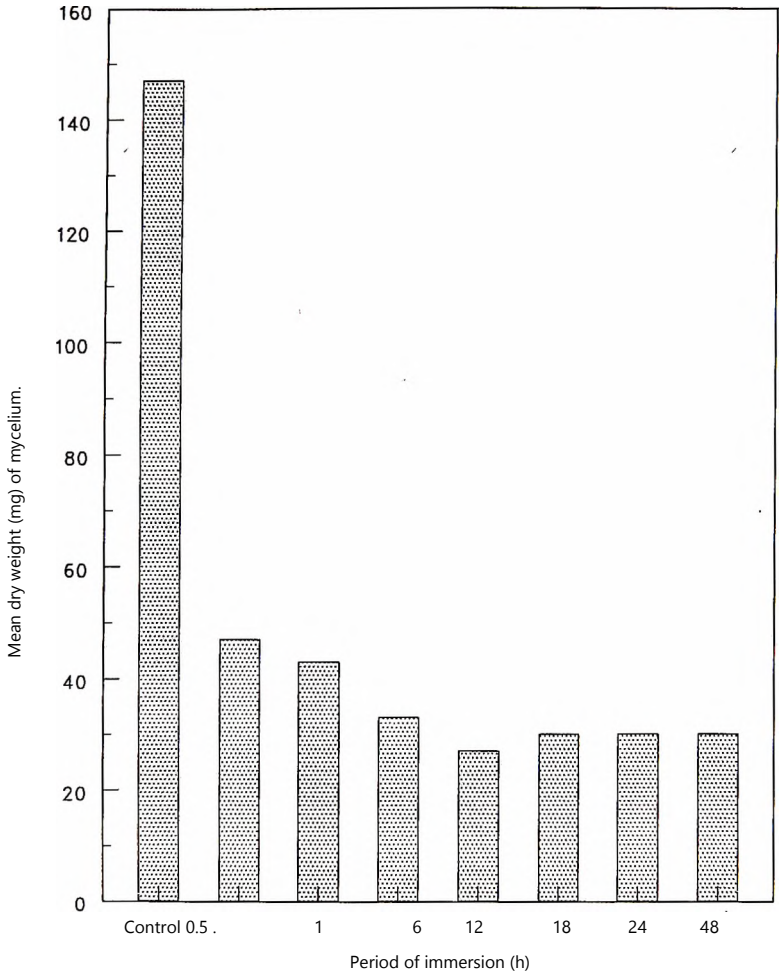
Vegetative growth of & rolfsii buried in undiluted methanol leaf extract of C. alata for varying periods before incubating in liquid medium at 28°C for 8days.



Methanol

Fig. 18

Vegetative growth of the mycelium of Helminthosporium sj). at 28°C in buried methanol extract of C. alata for varying periods in liquid medium.



Methanol

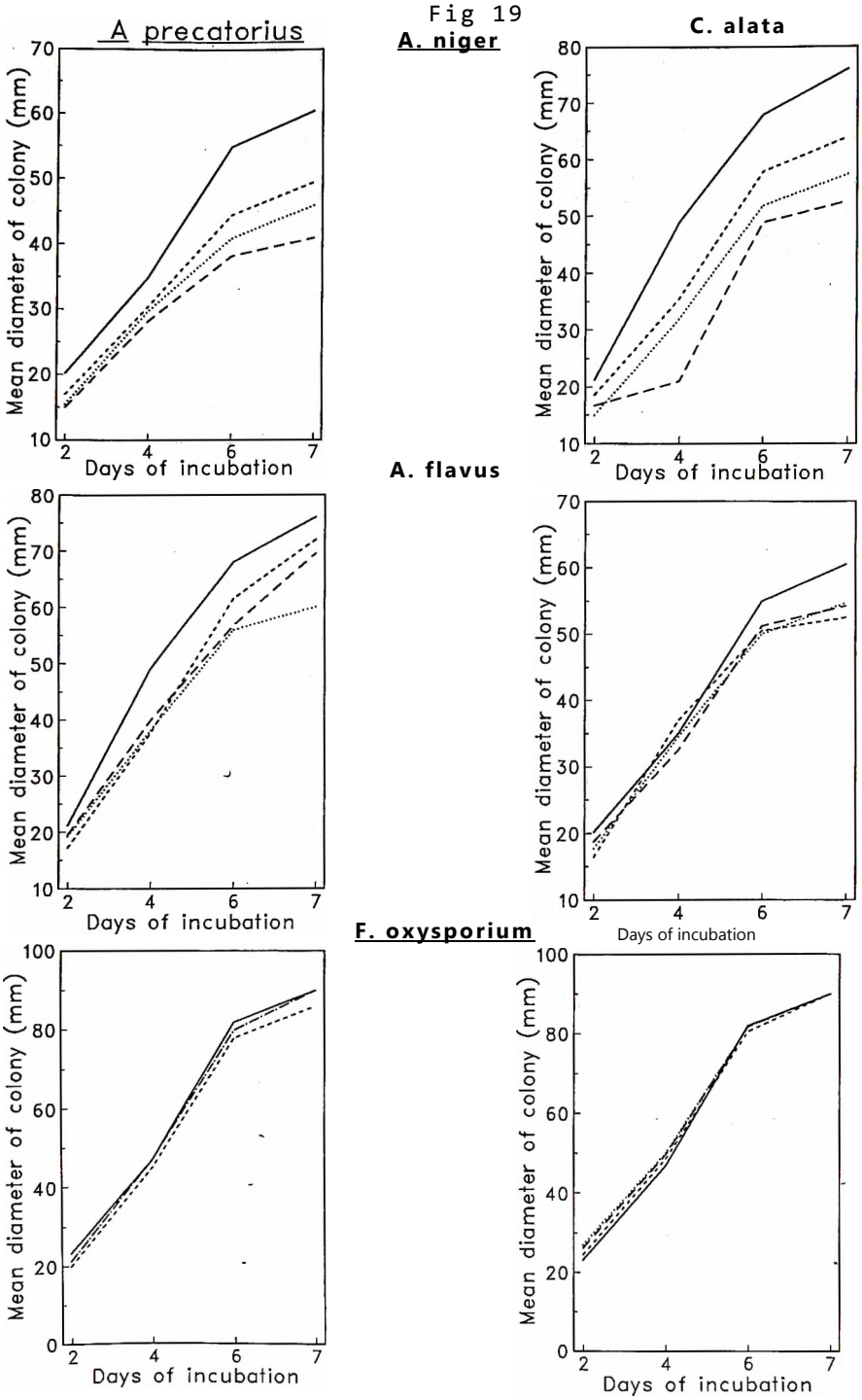
N.EFFECT OF THE LEAF EXTRACTS OF C. *ALATA* AND *A. PRECATORIUS* ON VEGETATIVE GROWTH OF *A. NIGER. A. FLAVUS* AND *F. OXYSPORIUM.*

The results obtained are presented in Figs.19 and 20 and in Appendices 48-49. There was significant difference between the vegetative growth of the test fungi as a result of treatments with the aqueous extracts of the dry leaves of C. alata and *A. precatorius.* For both extracts depression of growth on agar was in the order *A. niger* > *A. flavus* > *F. oxysporium.* The higher the concentration of the extract applied the greater the depression of growth observed Figs. 19 and 2 0.

Methanol extract.

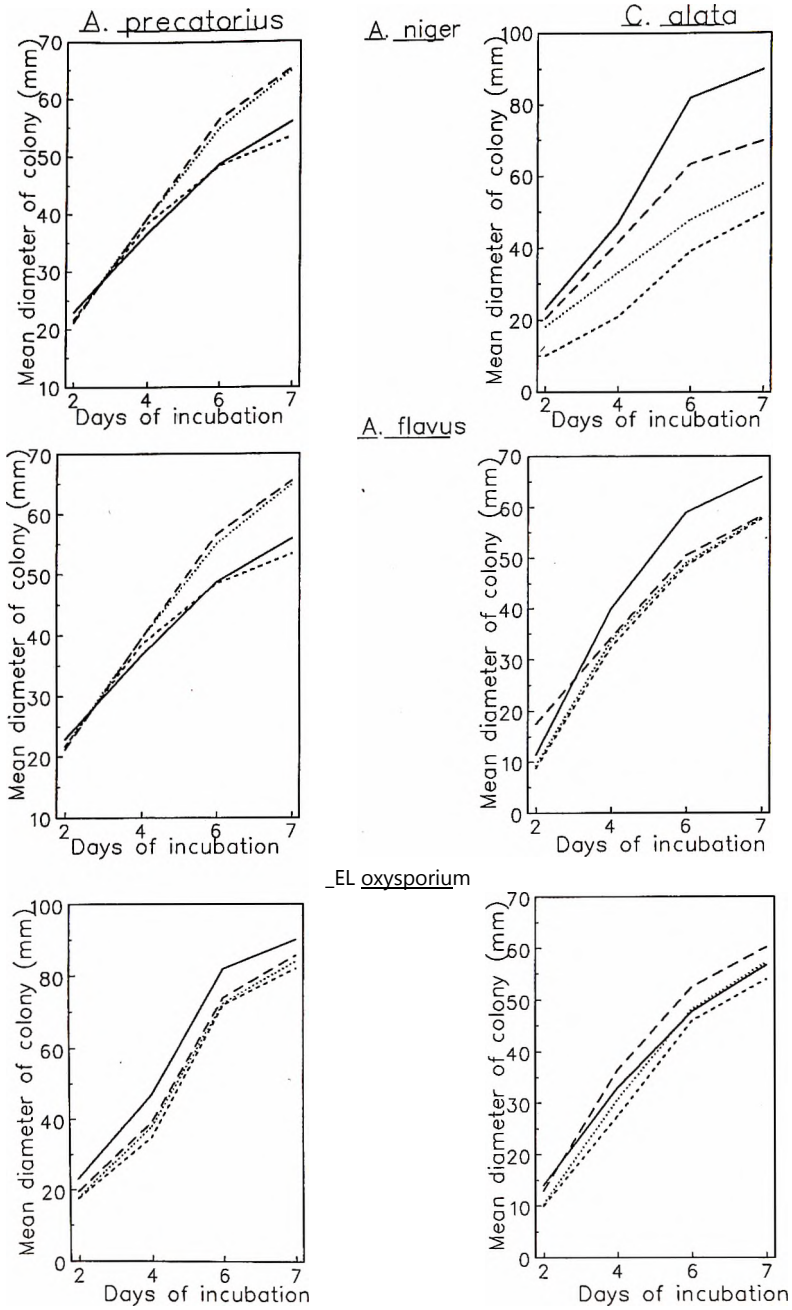
There was statistical difference between the vegetative growth of *A. niger, A. flavus* and *F. oxysporium* amended with methanol extracts of *C. alata* and *A. precatorius* and the control. Depression of vegetative growth was highest on *A. niger* and least on *F. oxysporium.* (Fig. 20).

Fig 19



Vegetative growth of niger, A. flavus and _F_. oxysporium in aqueous extracts of A^ precatorius and C.alata on agar at 28°C.

Fig. 20



Vegetative growth of A. niger. A. flavus and F. oxysporium in methanol extracts of A. precatorius and C. alata on agar at 28 C.

DISCUSSION

Naturally occurring antifungal and antibacterial compounds in plants have received much attention in recent years because of the increasing anxiety of the effect of agrochemicals on the ecosystem. Third World countries will benefit by an approach in which they can get a survey of possible control measures including biofungicides and biopesticides (Van Latum and Gerrits,1991).

The use of plants with toxic or repellant action against pest and pathogens is a common protection practice in traditional agricultural systems in developing countries. For example, the African savanna grass (*Hyparrhenia sp.)* produces a thermolabile, partially water-soluble toxin in soils in Ghana and Zimbabwe which is thought to inhibit *Nitrobacter* and especially *Nitrosomonas* in high-veld grassland that are very low in available nitrogen but not in nitrogen-fixing bacteria (*Clostridium, Azobacter* and *Beij erinckia spp.)* (Meiklejohn,1962; Clark and Paul, 1970) . Odamtten and Okyere (1990) showed that five non-fixing hosts of *Sclerotium rolfsii* namely *Commelina vulgelii* (Commelinaceae), *Euphorbia heterophylla* (Euphorbiaceae), *Manihot esculentus* (Euphorbiaceae), *Synedella nodiflora* (Compositae) and *Tridax procumbens* (Compositae) did not support growth of sclerotia placed on them and the sclerotia were recorded inviable at the end of 3 weeks incubation. Plants containing biotoxins can therefore be more interesting than synthetic chemical fungicides due to

inherent combinations of chemical substances. It is harder for fungi or insects to develop resistance to combinations of plant-derived chemicals than to single compounds. Therefore, natural biotoxins, when they have been tested carefully, may show great promise for use as natural biopesticides.

In Chapter A both aqueous and methanol extract of *Abrus precatorius, Cassia alata* and *Desmodium triflorum* depressed vegetative growth of *S. rolfsii* and *Helminthosporium sp.* (Fig 1 and 2; Appendix 1-6) . This extends the list of plants whose extracts depress growth of these fungi reported by Saksena and Tripathi (1986) and Apetorgbor (1991). These same extracts also significantly (P s 0.05) depressed sclerotia production (Fig 3 ; Table 4, Appendices 13-16). The degree of depression of sclerotia formation by the extracts can be ranked in descending order as follows:

Aqueous extract: *D. triflorum* > *C. alata* > *A. precatorius.*

The phytochemical contents of the plants listed in Table 1. indicates that *A. precatorius* contains abrine and abric acid and might have contributed to the relatively higher fungistasis observed.

The aqueous and methanol extracts of *Alternanthera pungens* (Amaranthaceae), *Sida acuta* (Malvaceae), *Boerhavia diffusa* (Nyctaginaceae) and *Oxalis corniculata* (Oxalidaceae) marginally depressed vegetative growth of both *S. rolfsii* and

Helminthosporium sp. in liquid medium and in some instances were completely ineffective (Fig 4-7) . On agar, depression did not exceed 66.35 percent in the highest concentration of the extracts (Table 5 and 6 ; Plate 2) . The effect of the inhibitory principles in the plants was severer on *Helminthosporium sp.* in most instances. Apetorgbor (1991) found the same marginal inhibitory effect of these plants on *Scopulariopsis brevicaulis, Nigrospora sp, A. flavus* and *S. rolfsii. 0. corniculata* however, showed the highest antibacterial activity against gram positive bacteria among eight plants tested by Apetogbor (1991) namely *Pergularia demia, Desmodium triflora, Alternanthera pungens, Voacanga africana. Cassia rotundifolia, Zanthoxylum xanthoxyloides* and *Azadirachta indica.* According to Gunasegaran (1992), the fresh leaf juice of *0. corniculata* cure dyspepsia, piles, anaemia and tympanitis. It also shows activity against gram positive bacteria. Phytochemical contents of the leaves of *0. corniculata* include vitamin C, carotene, citric and malic acid, C-glycosyl flavonoids [5,7,4-trihydroxy-8-C-B-D-gulcopyranoside (vitexin); 5,7,4'trihdroxy-6-C-D-glucopyranoside (isovitexin) and vitexin-2"-O-B-D-glucopyranoside] (Anon, 1966; Gunasegaran, 1992). The leaves are used for removing corns, warts and other excrescences of the skin; has antiseptic properties (Anon, 1966; Gunasegaran, 1992) and may find future use this country.

Interestingly, extracts of *A. repens, B. diffusa, 0. corniculata* and *S. acuta,* caused variable depressed sclerotia production by

S. rolfsii on agar; there was a corresponding decrease in size of the sclerotia (Table 7; Fig 8) . The effect was severest in methanol extracts of *A. repens* and least in *0. corniculata.* Presumably the active ingredient in *A. repens* (Table 1) with specifically interfere with metabolic process signal leading to sclerotia formation.

The inhibitory active principles in the aqueous and methanol extracts of *Azadirachta indica* (Meliaceae), *Clausena anisata* (Rutaceae) and *Zanthoxylum xanthoxyloides* (Rutaceae) (see Table 1) exerted marginal effect on the vegetative growth of *S. rolfsii* and *Helminthosporium sp.* at least at the highest concentration (1:1 v/v dilution) applied in both liquid and solid media (Chapters G and H) . The inhibitory effect was gradually removed with increasing dilution of the extracts. The fact that the minimal effect of the extracts on vegetative growth was significantly severer on *Helminthosporium sp.* connotes variation in susceptibility of the fungal species to the same "active principles" in the plant extracts.

In Chapter I, both aqueous and methanol dry leaf extracts of C. *anisata, Z. xanthoxyloides* depressed "in vitro" sclerotia formation by *S. rolfsii* (Fig 11; Table 10 and Appendices 35-38). The inhibitory effect was severest in extracts of C. *anisata.* Jacobson and Crosby (1971) and later Schmutter et *al* (1981) reviewed the literature on biocidal plants and included

C. anisata and *A. indica*, as candidate plants for control of insects. Findings in this thesis extends the spectrum of agents which could be controlled by these potential biocides.

The effect of the aqueous and methanol dry leaf extracts of *Catharanthus roseus* (Apocynaceae) and *Mitragyna inermis* (Rubiaceae) on vegetative growth of *S. rolfsii* can also be described as marginal (Chapters J and K) . Dilution of the extracts completely removed the inhibitory principles (Table 11 and 12; Figs 12 & 13).

The variations in susceptibility of the test fungi to the extracts was again confirmed (Tables 13, 14, 15 and 16) . *Helminthosporium* was more susceptible than *S. rolfsii* in most instances.

The alkaloid contents of *C. roseus* and *M. inermis* (see Table 1) at high concentrations (undiluted and 1:1 v/v dilution) variably and significantly ($P < 0.05$) depressed sclerotia formation (Fig 14 ; Table 17, Appendices 3 9 and 40) . The aqueous extract of C. *roseus* was more effective than the equivalent methanol extract (Table 17, Fig 14) while the vice versa was true for *M. inermis* at least at the highest concentration applied (Fig 14) . Variation in yield of active ingredient from plants depending on the nature of the solvent used has been demonstrated by Rao and Ahamed (1992) for *Dalbergia paniculata* leaves. They found that light petrol extract yielded triacontanol and B-sistosterol. Acetone extraction gave (+)-pinitol, apigenin, luteolin and 7-0-glucosycaviunin and methanol extraction yielded 4,7-di-O-

glucosylavpigenin (Rao and Ahamed 1992). As data accumulates on potential plants with fungicidal and bactericidal action in this laboratory, it may be expedient to expand the spectrum of solvents used in the extraction process. Time limitation prevented such investigations to be carried out in this thesis. However, this could form the basis of a separate study which will be reported in another thesis from this laboratory.

An information of practical importance is the period for which viability of the fungus would be sustained in the presence of the fungistatic principles in the extract. The shorter the period to "inactivate" the fungus, the greater the usefulness of the extract as a biological control agent. In Chapter M, it required a minimum immersion period of 3 0 minutes in the leaf extract of *Cassia alata* to impair vegetative growth of *S. rolfsii* and *Helminthosporium sp.* The longer the period of immersion, the severer the depression in the dry weight of mycelium (Fig 17 and 18). Methanol extract was more effective than the aqueous extract. Furthermore, both vegetative growth and sclerotia production by *S. rolfsii* was completely prevented when the mycelium was buried in methanol extract of C. *alata* for 48hrs. prior to transfer unto extract-free agar medium; vegetative growth of *Helminthosporium sp.* similarly treated was depressed by 20.49 percent. In the concluding Chapter of this thesis, *C. alata* and *A. precatorius* extracts also variably depressed vegetative growth of *Aspergillus niger, A. flavus* and *Fusarium oxysporium.*

C. alata is employed in traditional medicine in the treatment of parasitic skin diseases such as itch, eczema, ringworm; it has been recorded to exhibit anti-inflammatory, antifungal, wound healing and antibacterial activates against *Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeroginosa, Escherichia coli, Candida albicans, Trichophyton mentagrophytes, Penicillium italicum, Aspergillus fumigatus* and *Lasilioplodia theobromae* (Ogunti et al. 1991) . The antifungal and antibacterial activities of the leaves have been traced to the presence of rhein, B-sitosterol, kaempferol, amino acids, coelutatin and azulene (Ogunti *et al.* 1991). Findings in this thesis extends the list of fungi susceptible to this plant extract and also validates the use of *C. alata* in African ethnomedicine and future use in the control of plant pathogenic fungi.

The general conclusions that can derive from these studies is that both the aqueous and methanol extract of the test plants had variable and appreciable depression effect on the vegetative growth of *S. rolfsii, Helminthosporium sp. A. niger, A. flavus* and *F. oxysporium.* The effect was severer on *Helminthosporium sp.* The extracts however were very effective in reducing "in vitro" sclerotia formation by *S. rolfsii.* Promising plants include C. *alata, Abrus precatorius, 0. corniculata* to mention but a few. The minimum period of contact with the fungus to reduce or inactivate vegetative growth varied from 30 minutes to 48 hours.

Plant biotoxins are natural toxins that need to be scrutinised carefully because possible harm may result from overexposure to some of such substances just as it is with certain synthetic chemicals. To achieve a higher level of food self-sufficiency, it is generally recognised that low-input agricultural methods are important for both home consumption and local market. Agroforestry systems often combine traditional agricultural techniques with production of plants from which biotoxins are derived (Van Latum and Gerrits, 1991). These farming systems contribute to a low cost, secure, better and higher yielding agriculture crop.

Biopesticides/biofungicides are therefore of potential importance for low-input farming. But because the substances derived from them can sometimes be toxic, they cannot be applied indiscriminately. With the help of research under controlled circumstances, criteria and guidelines can be developed under which biofungicides can be promoted further and at some time their application be rendered environmentally friendly.

SUMMARY.

1. The fungicidal and fungistatic potential of 12 Ghanaian plants namely *Abrus precatorius* (Papilionaceae) , *Alternanthera pungens (repens)* (Amaranthaceae), *Azadirachta indica* (Meliaceae), *Boerhavia diffusa* (Nyctaginaceae) , *Cassia alata* (Caesalpinaceae), *Catharanthus roseus* (Apocynaceae), *Clausena anisata* (Rutaceae), *Desmodium triflorum* (Papilionaceae), *Mitragyna inermis* (Rubiaceae), *Oxalis corniculata* (Oxalidaceae) , *Sida acuta* (Malvaceae) and *Zanthoxylum xanthoxyloides* (Rutaceae) have been tested against S. *rolfsii* and *Helminthosporium sp.* in vitro.

2. Comparative higher fungistasis against *S. rolfsii* and *Helminthosporium sp* ./were found in both aqueous and methanol extract of the dry leaves of *C. alata, Abrus precatorius* and *Desmodium triflorum.*

3. C. *alata* methanol extract of 1:1, 1:2 and l:the/v depressed vegetative growth by 75.79, 63.25 and 14.83 percent respectively; same concentration of *D. triflorum* depressed vegetative growth up to 46.89, 19.27 and 16.71 percent respectively.

4. Extract dilution of l:lv/v of *A. precatorius* methanol extract depressed vegetative growth of test fungi up to 45.27 percent.

5. The remaining plants {*A. indica, A. pungens, B. diffusa, C.*

roseus, C. anisata, S. acuta, M. inermis, O. corniculata and Z. xanthoxyloides) exerted minimal inhibitory effect on the test fungi.

6.Generally the inhibitory effect, even if minimal was severer on *Helminthosporium sp.* than *S. rolfsii.*

7. Sclerotia production by *S. rolfsii* on agar amended by extracts of the test plants was variable but significantly depressed by all the test plants especially at high concentrations of 1:1 - 1:2v/v dilution. The inhibitory effect was gradually removed with increasing dilution of the extracts.

8. The sizes of the surviving sclerotia produced in the methanol extract of *A. precatorius* did not differ significantly (P s 0.05) from that of *C. alata.*

9. The effectiveness of the l:lv/v dilution of the aqueous extract of plants in the families Amaranthaceae, Malvaceae, Nyctaginaceae and Oxalidaceae on sclerotium production can be ranked as follows in descending order: *S. acuta* (83.78%) > *B. diffusa* (56.77%) > *O. corniculata* (40.53%) > *A. repens* (36.16%).

10. The inhibitory effect of the plant extracts on vegetative growth of the test fungi were gradually removed with increasing dilution.

11. The effect of the aqueous extract of Z. *xanthoxyloides* was severer on *S. rolfsii* than on *Helminthosporium sp.*

12. The aqueous extract of *C. anisata* showed no significant difference between the vegetative growth of *S. rolfsii* and *Helminthosporium sp..*

13 . The methanol extract of *M. inermis* suppressed the vegetative growth of *S. rolfsii* more than *Helminthosporium sp.* on solid medium.

14. The aqueous extracts of *B. diffusa, C. roseus 0. corniculata* and *S. acuta* were more depressive in their effect on sclerotia production by *S. rolfsii* than the equivalent methanol extract.

15. The depressive effect of the extracts on the number and dimensions of sclerotia formed were gradually removed with increasing dilutions of the extracts.

16. The depression on sclerotia production was attended by a corresponding increase in their dimensions although the trend was not as straight forward as for the decline in numbers.

17. It required a minimum immersion period of 3 0 minutes in the methanol leaf extract of *C. alata* to impair the vegetative growth of the mycelium of *3. rolfsii* and *Helminthosporium sp.*

18. The methanol extract of *C. alata* depressed vegetative growth of the test fungi by 70-80 percent depending on the period of immersion, the longer the period the severer the depression in the dry weight of the harvested mycelium.

19. On agar both vegetative and sclerotia production by *S. rolfsii* was completely prevented when the mycelium was buried in the methanol extract of *C. alata* for 48h prior to inoculating on extract-free medium.

20. Extracts of *A. precatorius* and *C. alata* significantly suppressed the vegetative growth of *A. niger, A. flavus* and F. *oxysporium* at (P s 0.05) . Depression of growth was in the order *A. niger > A. flavus > F. oxysporium.*

Appendix 1a

Analysis of Variance showing the effect of the methanol extract of _D. triflorum on the vegetative growth of S. rolfsii and Helminthosporium sp. In liquid medium.

Source of variation	Sum of Squares	d.f.	Mean square	Cal. F-ratio	F—rafio(Table)
MAIN EFFECTS	139,825	7	19,974,93000	126.462	
fungi	120,050	1	120,050.0000	760.039	4.17
dilution	19,4745	3	6,491.50000	41.098	2.92
replicates	300	3	100.0000	0.633	
2-FACTOR INTERACTION	16,785	3	5,594,83300	35.421	2.92
fungi and dilution	16,785	3	5,594,83300	35.421	2.92
RESIDUE	3,317	21	157.95238		
TOTAL (CORR)	159,926	31			

Appendix 1b

Multiple range analysis showing the effect of the methanol extract of *D.* triflorum on fungal growth on agar.

Fungus	Count	Average	Homogenous Group
Helminthosporium sp. 16		55.00	A
S. rolfsii	16	177.50	B

Figures with different letters are significantly different.

Appendix *2a*

Analysis of Variance showing the effect of the methanol extract of JL orecatorlus on the vegetative growth of IL rolfsii and Helminthosporium sp* In liquid medium.

Source of variation	Sum of Squares	d.f.	Mean square	Cat. F-ratio	F—ratio(Table)
MAIN EFFECTS	157,763,250	7	2,253.32140	18.638	
fungi	112.500	1	112.50000	0.931	4.17
dilution	15,610.125	3	5,203.3750	43.039	2.92
replicates	50.625	3	16.8750	0.140	
-FACTOR INTERACTION	2,510.750	3	836.91667	6.922	2.92
fungi and dilution	2,510.750	3	836.91667	6.922	2.92
RESIDUE	2,538.875	21	120.89881		
TOTAL (CORR)	20,822.875	31			

Appendix 2b

Multiple range analysis showing the effect of the methanol extract of fungal growth on agar.

Fungus Count	Average	Homogenous Group
HelminthosDorium sp. 16	**106.4375**	**A**
S. rolfsii 16	**110.1875**	**B**

Figures with different letters are significantly different. -

Appendix 3a

Analysis of Variance showing the effect of the methanol extract of C. alata on the vegetative growth of S. rolfsii and Helminthosporium sp. In liquid medium.

Source of variation	Sum of Squares	d.f.	Mean square	Cal. F-ratio	F-ratio(Table)
MAIN EFFECTS	87,056.21900	7	12,436,6030	416.765	
fungi	5,644.53100	1	5,644.5310	189.155	4.17
dilution	1,308.59400	3	27,102.8650	908.249	2.92
replicates	103.0940	3	34.3650	1.152	
-FACTOR INTERACTION	2,559.09380	3	853.03125	28.586	2.92
fungi and dilution	2,55.09380	3	853.03125	28.586	2.92
RESIDUE	626.65625	21	29.84077		
TOTAL (CORR)	90,241.9690	31			

Appendix 3b

Multiple range analysis showing the effect of the methanol extract of C. alata on fungal growth on agar.

Fungus	Count	Average	Homogenous Group
Helminthosporium sp. 16		84.2500	A
S. rolfsii	16	110.8125	B

-Figures with different letters are significantly different.

Appendix 4a

Analysis of variance showing the effect of the aqueous extract of triflorum on the vegetative growth of S. rolfsii and Helminthosporium sp. in liquid medium.

Source of variatio	Sum of squares	d.f	Mean square	Cal.F—ratio	Table F—ratio
Main Effect	108,082.880	7	15,440.41000	250.797	
fungi	100,128.130	1	100,128.13000	1,000.000	4.17
Dilution	7,739.130	3	2,579.71000	41.902	2.92
Replicates	215.530	3	71.88000	1.167	2.92
2-Factor Interaction	2,249.125	3	749.70833	12.177	
fungi and dilution	2,249.125	3	749.70833	12.177	2.92
Residual	1,292.875	21	61.56548		
Total(corr)	111,624.880	31			

Appendix 4b

Multiple range analysis showing the effect of the aqueous extract of D. triflorum on fungal growth in liquid medium.

Fungus	Count	Average	Homogenous Group
Helminthosporium sp.	16	54.375	A
S. rolfsii	16	166.250	B

Figures with different letters are significantly different.

Appendix 5a

Analysis of variance showing the effect of the aqueous extract of A. precatorius on the vegetative growth of S. rolfsii and Helminthosporium sp. in liquid medium.

Source of variation	Sum of squares	d.f	Mean square	Cal.F-ratio	Table F—ratio
Main Effect	75,989.00	7	10,855.57100	242.196	
Fungi	67,344.50	1	67,344.50000	1,000.000	4.17
Dilution	8,583.25	3	2,861.08300	63.833	2.92
Replicates	61.25	3	20.41700	0.456	2.92
2—Factor Interaction	19,945.25	3	6,648.41670	148.331	
Fungi and dilution	19,945.25	3	6,648.41670	148.331	2.92
Residual	941.25	21	44.82314		
Total(corr)	96,875.50	31			

Appendix 5b

Multiple range analysis showing the effect of the aqueous extract of A precatorius on fungal growth in liquid medium.

Fungus	Count	Average	Homogenous Group
Helminthosporium sp.	16	74.00	A
S. rolfsii	16	165.75	B

Figures with different letters are significantly different.

Appendix 6a

Analysis of Variance showing the effect of aqueous extract of C. alata on the vegetative growth of S. rolfsii and Helminthosporium sp. in liquid medium.

Source of variation	Sum of Squaresd.f.		Mean square	Cal. F—ratio	F—ratio (Table)
MAIN EFFECTS	22,040.21900	7	3,148.60300	' 82.096	
x fungi	11,666.28100	1	1 1,66.28100	N 304.184	4.17
dilution	10,229.594^00	3	3,409.86500	88.908	2.92 N
replicates	144.34400	3	48.11500	1.255	
2-FACTOR INTERACTION	28,927.84400	3	9,642.61460	251.420	2.92
fungi and dilution	28,927.8400	3	9,642.61460	251.420 -	2.92
RESIDUE	805.40625	21	38.35268		
TOTAL (CORR>)	51,773.46900	31			

Appendix 6b

Multiple range analysis showing the effect of the aqueous extract of C. alata on fungal growth in liquid medium.

Fungus	Count	Average Homogenous Group		
Helminthosporium sp. '	16	120.125	A	
S. rolfsii	16	158.312		B

Figures with different letters are significantly different.

Appendix 7a

Analysis of Variance showing the effect of the methanol extract of D. triflorum on the vegetative growth of S. rolfsii and Helminthosporium sp. on agar.

Source of variation]S	um of Squares	d.f.	Mean square	Calculated F—ratio	F—ratio (Table)
MAIN EFFECTS	15,870.21900	7	2,267.17400	222.577	
fungi -s	13,571.28100	1	13,571.28100	1,000.000 ^	4.17
dilution	2,294.59400	3	764.86500	75.090	2.92
replicates	4.34400	3	1.44800	0.142	
2-FACTOR INTERACTION	39.34375	3	13.11458	1.288	
fungi and dilution	39.34375	3	13.11458	1.288	2.92
RESIDUAL	213.90625	21	10.18601		
TOTAL (CORR>)	16,123.46900	31			

Appendix 7b

Multiple range analysis showing the effect of the methanol extract of D. triflorum on fungal growth on agar.

Fungus	Count	Average	Homogenous	Group
Helminthosporium sp.	16	37.1875	- A	
S.'rolfsii	16	78.3750	'	B

Figures with different letters are significantly different.

Appendix 8a

Analysis of Variance showing the effect of the methanol extract of A.precatorius on the vegetative growth of S. rolfsii and Helminthosporium sp. on agar.

Source of variation	Sum of Squares	d.f.	Mean square	Calculated F-ratiifr—ratio (Table)	
MAIN EFFECTS	'' 11.088.71900	7	1,584.10270	363.937	
fungi	N 7411.531	1	7,411.53130	1,000.000	N 4.32
dilution	3,641.84400	3	1,21^.94790	278.897	3.07
replicates	35.34400	3	11.78130	2.707	
2-FACTOR INTERACTION	128.09375	3	42.69792	9.810	3.07
fumgi and dilution	128.09375	3	42.69792	9.810	3.07 -
RESIDUE	91.40625	21	4.35268		
TOTAL (CORR>)	1 1,308.21900	31			

Appendix 8b

Multiple range analysis showing the effect of the methanol extract of A. precatorius on fungal growth on agar.

Fungus	Count	Average	Homogenous Group
Helminthosporium sp.	16	38.9375	A
S. rolfsii	16	69.3750	B

Figures with different letters are not significantly different.

Appendix 9a

Analysis of Variance showing the effect of methanol extract of C. alata on the vegetative growth of S. rolfsii and Helminthosporium sp. on agar.

Source of variation	Sum of Squares	d.f.	Mean square	Cal. F—ratio	F—ratio (Table)
MAIN EFFECTS	17,143.125	7	2,449.01790	132.507	
species	2,775.125	1	2,775.12500	150.152	4.32
dilution	14,357.625	3	4,785.87500	285.946	3.07
replicates	10.375	3	3.45830	0.187	
2-FACTOR INTERACTION	490.625	3	163.54167	8.849	3.07
species and dilution	490.625	3	163.54167	8.849	3.07
RESIDUE	388.125	21	18.482143		
TOTAL (CORR>)	18,020.875	31			

Appendix 9b

Multiple range analysis showing the effect of the methanol extract of C. alata on fungal growth on agar.

Fungus	Count	Average	Homogenous Group
Helminthosporium sp.	16	20.750	A
S. rolfsii	16	39.375	B

Figures with different letters are significantly different.

Appendix 10a

Analysis of variance showing the effect of the aqueous extrgct of D. triflorum on the vegetative growth of S. rolfsii and Helminthosporium S£. on agar.

Source of variation	Sum of Squares	d.f.	Mean squar£	F—ratio	F—ratio (Table)
MAIN EFFECTS	6400.6694	7	814.3813	1000.000	
fungi	5755.3200	T	5755.3200	1000.000	4.32
dilution	612.3333	3	204.1111	296.889	3.07
replicates	3.5093	3	1.1698	1.701	
2-FACTOR INTERACTION	2041.0000	3	680.33333	989.576	3.07
Fungi and dilution	2041.0000	3	680.33333	989.576	3.07
RESIDUE	13.750000	21	0.6875000		
TOTAL (CORR>)	8455.4194	31			

Appendix 10b

Multiple range analysis showing the effect of the aqueous extract D. triflorum on fungal growth.

Fungus	Count	Average	Homogenous	Group
Helminthosporium sp.	16	59.666667	A	
S. rolfsii	16	87.000000		B

Figures with different letters are significantly different.

Appendix 1 1 a

Analysis of Variance showing the effect of the aqueous extract of A.precatorlus on the vegetative growth of S. rolfsii and Helminthosporium sp. on agar.

Source of variation	Sum of Squares	d.f.	Mean square	Cal. F—ratio	Sig.level	F-ratio(Tal
MAIN EFFECTS	10,926.96900	7	1,560.99550	512.232	0.0000	
fungi	4,413.30100	1	4,413.30120	1,000.000	0.0000	4.32
dilution	6,499.75400	3	2,166.58460	710.000	0.0000	3.07
replicates	13.91400	3	4.63790	1.520	0.2381	
-FACTOR INTERACTION	719.80375	3	239.93458	78.733	0.0000	3.07
fungi and dilution	719.80375	3	239.93458	78.733	0.0000	3.07
RESIDUE	63.99625	21	3.04.744			
TOTAL (CORR)	1 1,710.76900	31				

Appendix 11b

Multiple range analysis showing the effect of the aqueous extract of A. precatorius on fungal growth on agar.

Fungus	Count	Average	Homogenous Group
Helminthosporium sd.	16	36.1250	A
S. rolfsii	16	59.6125	B

Figures with different letters are significantly different.

Appendix 12a

Analysis of variance showing the effect of the aqueous extract of C. alata on the vegetative growth of S. rolfsii and Helminthosporium sp. on agar.

Source of variation	Sum of Squares	d.f.	Mean square	Cal. F-ratio	F-ratio (Table)
MAIN EFFECTS	8,309.27870	7	1,187.03980	14.836	
fungi	2,174.70120	1	2,174.70120	27.180	4.32
dilution	5,875.00370	3	1,958.33460	24.476	3.07
replicates	259.57370	3	86.52460	1.081	
2-FACTOR INTERACTION	76.30375	3	25.43458	0.318	3.07
Fungi and dilution	76.30375	3	25.43466	0.318	3.07
RESIDUE	1,680.21630	21	80.01030		
TOTAL (CORR)	10,065.79900	31			

Appendix 12b

Multiple range analysis showing the effect of the aqueous extract of A. precatorius on fungal growth on agar.

Fungus	Count	Average	Homogenous Group	
Helminthosporium sp.	16	42,7500	A	
S. rolfsii	16	59.2375		B

Figures with different letters are significantly different.

Appendix 13a

Analysis of Variance table showing the number of sclerotia formed by the aqueous extracts of A. precatorius. C. alata and D. triflorum on agar.

Source of variation	Sum of Squares	d.f.	Mean square	Cal. F-ratio	F-ratio (Table)
MAIN EFFECTS	487188.50	8	60898.56	188.367	
species	54782.00	2	27391.00	84.724	3.3
dilution	431885.33	3	143961.78	445.291	2.9
replicates	521.1700	3	173.7200	0.537	
2-FACTOR INTERACTION	53876.667	6	8979.4444	27.775	2.40
species and dilution	53876.667	6	8979.4444	27.775	2.4
RESIDUAL	10668.833	33	323.29798		
TOTAL (C0RR>)	551734.00	47			

Appendix 13b

Multiple range analysis of the effect of aqueous extracts of indicated plants on sclerotia production.

Plant species	Count	Average	Homogenous Group
A. precatorius	16	9 1.750	A
Cl. alata	16	132.875	B
D. triflorum	16	174.500	C

Figures with different letters are significantly different.

Appendix 14a

Analysis of variance table showing the number of sclerotia formed by the methanol extracts of species presented in table 4 .

, Source of vqj-iation	Sum of squares	d.f	Mean square	Cal.F-ratio	Table F-ratio
Main Effect	1125761.7	8	140720.21	302.308	
Species	825163.0	2	412581.52	886.347	3.3
Dilution	299204.9	3	99734.97	214.260	2.9
Replicates	1393.7	3	464.58	0.998	
2-Factor Interaction	261303.79	6	43550.632	93.560	2.4
Species and Dilutio	261303.79	6	43.550.632	93.560	2.4
Residual	15361.021	33	465.48548		
Total(corr)	1402426.5	47			

Appendix 14b

Multiple range analysis of the methanol extract of indicated plants on sclerotia production.

Plant species	Count	Average	Homogenous Group
H, alata	16	74.87500 _	A
A. precatorius	16	92.43750	A
D. triflorum	16	361.37500	B

Figures with the same letters are not significantly different.

Appendix 15a

Analysis of variance of data in table 4 showing effect of aqueous extract of test plants on sizes of sclerotia produced.

Source of variation	Sum of squares	d.f	Mean squareCal.F—ratio		Table F-ratio
Main Effect	N 206 1.2403	8	257.65504	9.202	X
Species	715.1937	2	357.59683	12.772	3.3
Dilution	1237.6793	3	412.55976	14.735	2.9
Replicates	108.3674	3	36.12247	1.290	
2—Factor Interaction	1052.0450	6	175.34083	6.262	2.4
Species and Dilutio	1052.0450	6	175.34083	6.262	2.4
Residual	923.96112	33	27.998822		
Total(corr)	4037.2464	47			

Appendix 15b

Multiple range analysis of the data in table 4 showing the effect of aqueous plant extracts on size of sclerotia.

Plant species	Count	Average	Homogenous Groups
C. alata	16	77.300625	A
A. precatorius	16	78.442500	A
D. triflorium	16	86.000000	8

Fiaures with the same letters are not significantly different.

Appendix 16a

Analysis of variance of data in table 4 showing the effect of test plants on the sizes of sclerotia formed.

Source of variation	Sum of squares N	d.f	Mean square	Cal.F—ratio	Table' F-ratio
Main Effect	2689.421 1	8	336.17764	"K9.445	
Species	412.4436	2	206.22181	11.928	3.3
Dilution	2266.2406	3	755.41353	43.694	2.9
Replicates	10.7369	3	3.57898	0.207	
2-Factor Interaction	606.18439	6	101.03073	5.844	2.4
Species and Dilution	606.18439	6	101.03073	5.844	2.4
Residual	570.53221	33	17.288855		
Total(corr)	3866.1377	47			

Appendix 16b

Multiple range analysis showing the effect of methanol extracts of indicated plants on the sizes of sclerotia.

Plant species	Count	Average	Homogenous Groups
C. alata	16	71.631250	A
A. precatorius	16	72.560625	A
D. triflorium	16	78.261875	B

Figures with the same letters are not significantly different.

Appendix 17a

Multiple range analysis of the effect of aqueous extract of A. repens on the vegetative growth of Indicated fungi In liquid medium.

Fungus	Count	Average	Homogeneous Group
HelminthosDorium sd.	16	127.93750	**A**
S. rolfsii	16	153.25000	**A**

Appendix 17b

Multiple range analysis of the effect of aqueous extract ofJL diffusa on the vegetative growth of indicated fungi in liquid medium. (Note differences between Scheff Averages for both fungi.)

Fungus	Count	Average	Homogeneous Group
HelminthosDorium sp.	16	123.56250	**A**
S. rolfsii	16	136.25000	B

Figures with the same letters are not significantly different.

Appendix 18a

Multiple range analysis of the effect of the methanol extract of A. repens on the vegetative growth of indicated fungi in liquid medium.(Note differences between Scheff Averages for both fungi.)

F ungus	Count	Average	Homogeneous Group
Helminthosporium sp.	16	101.875	A
S. rolfsii	16	129.375	B

Appendix 18b

Multiple range analysis of the effect of methanol extract of_B. diffusa on the vegetative growth of indicated fungi in liquid medium. (Note differences between Scheff Averages for both Fungi).

Fungus	Count	Average	Homogeneous Group
Helminthosporium sp.	16	49.5625	A
S. rolfsii	16	168.3125	B

Figures with different letters are significantly different.

Appendix 19a

Multiple range analysis of the effect of aqueous extract of O, corniculata on the vegetative growth of indicated fungi in liquid medium.

Fungus	Count	Average	Homogeneous Group
Helminthosporium sp.	16	130.0	**A**
S. rolfsii	16	145.0	**A**

Appendix 19b

Multiple range analysis of the effect of aqueous extract of jL acuta on the vegetative growth of indicated fungi in liquid medium. (Note differences between Scheff Averages for both Fungi).

Fungus	Count	Average	Homogeneous Group	
HelminthosDorium sp.	16	114.1875	**A**	
S. rolfsii	16	163.125		**B**

F'igures with different letters are significantly different.

Appendix 20a

Multiple range analysis of the effect of methanol extract of Q. corniculata on vegetative growth of indicated fungi in liquid medium. (Note differences between Scheff Averages for both fungi).;

Fungus	Count	Average	Homogeneous Group
Helminthosporium sp.	16	122.50000	**A**
S. rolfsii	16	124.37500	**A**

Appendix 20b

Multiple range analysis of the effect of methanol extract of JL acuta on the vegetative growth of indicated fungi in liquid medium. (Note differences between Scheff Averages for both Fungi).

Fungus	Count	Average	Homogeneous Group
Helminthosporium sp.	16	63.31250	**A**
	16	144.18750	**B**

Figures with the same letters are not significantly different.

Appendix 21a

Multiple range analysis of aqueous extract of B.diffusa on the vegetative growth of indicated fungi on agar. (Note differences between Scheff Averages for both fungi).

Fungus	Count	Average	Homogeneous Group
HelminthosDorium sd.	16	76.3125	A
S. rolfsii	16	90.0000	B

Appendix 21b

Multiple range analysis of methanol extract of B.diffusa on the vegetative growth of indicated fungi on agar medium. (Note differences between Scheff Averages for both Fungi).

Fungus	Count	Average	Homogeneous Group
Helminthosporium so.	16	69.8125	A
S. rolfsii	16	90.0000	B

Figures with different letters are significantly different.

Appendix 22a

Multiple range analysis of aqueous extract of S.acuta on the vegetative growth of indicated fungi on agar. (Note differences between Scheff Averages for both fungi).

Fungus	Count	Average	Homogeneous Group
HelminthosDorium SD.	16	70.4375	A
S. rolfsii	16	86.7500	B

Appendix 22b

Multiple range analysis of methanol extract ofJL acuta on the vegetative growth of indicated fungi on agar medium. (Note differences between Scheff Averages for both Fungi).

Fungus	Count	Average	Homogeneous Group
Helminthosporium sp.	16	53.2500	A
S. rglfsii	16	79.3125	B

Figures with different letters are significantly different.

Appendix 23a

Multiple range analysis of aqueous extract of A. repens on the vegetative growth of indicated fungi on agar (Note differences between Scheff Averages for both fungi).

Fungus	Count	Average	Homogeneous Group
HelminthosDorium sp.	16	65.562500	
S. rolfsii	16	90.000000	**B**

Appendix 23b

Multiple range analysis of methanol extract of A. repens on the vegetative growth of indicated fungi on agar (Note differences between Scheff Averages for both fungi).

Fungus	Count	Average	Homogeneous Group
HelminthosDorium sp.	16	23.625000	**A**
S. **rolfsii**	16	45.875000	**B**

Figures with different letters are significantly differently.

Appendix 24a

Multiple range analysis of aqueous extract of O.corniculata on the vegetative growth of indicated fungi on agar. (Note differences between Scheff Averages for both fungi).

Fungus	Count	Average	Homogeneous Group
HelminthosDorium sd.	16	56.125	**A**
S. rolfsii	16	86.250	**B**

Appendix 24b

Multiple range analysis of methanol extract of O. corniculata on the vegetative growth of indicated fungi on agar medium. (Note differences between Scheff Averages for both Fungi).

Fungus	Count	Average	Homogeneous Group
HelminthosDorium sp.	16	64.75	**A**
S. rolfsii	16	90.00	**B**

Figures with different letters are significantly different.

Appendix. 25a

Analysis of variance of data showing the number of sclerotia formed in the aqueous extracts of species presented in table 7.

Source of variation	Sum of Squares	d.f	Mean square	Cal.F-ratio	Table F-rafio
MAIN EFFECTS	609799.64	9	67755.52	892.707	
species	192953.17	3	64317.72	847.412	2.85
dilution	416687.17	3	138895.72	1000	2.85
replicates	159.30	3	53.10	0.700	
2 FACTOR INTERACTIONS	49193.516	9	5465.9462	72.016	2.10
species and dilution	49193.516	9	5465.9462	72.016	2.10
RESIDUE	3415.4531	45	75.898958		
TOTAL (CORR)	662408.61	63			

Appendix 25b

Multiple range analysis for the data presented in table 7 showing the effect of aqueous extract of the indicated plants on sclerotia formation.

Species	Count	Average	Homogenous Groups
S. acuta	16	220.000	A
B. diffusa	16	265.750	B
0. corniculata	16	329.625	C
A. reDens	16	361.125	D

Figures with different letters are significantly different.

Appendix 26a

Analysis of variance for the sizes of sclerotia formed in the aqueous extracts of plants shown in Table 7.

Source of variation	Sum of squares	d.f	Mean square	Cal.F—ratio	Table F—ratio
Main Effect	3459.3497	9	384037218	33.766	
Species	1685.8275	3	561.94250	49.365	2.85
Dilution	1690.9367	3	563.64555	49.514	2.85
Replicates	82.5855	3	27.52850	2.418	
2-Factor Interaction	1899.4013	9	211.04459	18.539	2.10
Species and Dilution	1899.4013	9	211.04459	18.539	2.10
Residual	512.25859	45	11.383524		
Total(corr)	5871.0096	63			

Appendix 26b

Multiple range analysis for data presented in table 7 on the effect of aqueous extract of the indicated plants on the sizes of sclerotia production by JL lolfsii

Plants species Count	Average	Homogenous Groups		
B. diffusa 16	67.40	A		
O. corniculata 16	68.9575	A		
A. repens 16	74.141875		B	
S. acuta 16	80.523125			C

Figures with the same letters are not significantly different.

Appendix 27a

Analysis of variance showing the number of sclerotia formed in methanol extracts of species presented in table 7.

Source of variation	Sum of Squares	d.f.	Mean square	Cal. F—ratio	F—ratio (Table)
MAIN EFFECTS	950,306.3900	9	105,589.60000	1,000.000	
species	571,119.4200	3	190,373.14000	1,000.000	2.85
dilution	379,141.4200	3	126,380.47000	1,000.000	2.85
replicates	45.5500	3	15.18000	0.189	
-FACTOR INTERACTION	178,174.7700	9	19,797.19600	246.424	2.10
species and dilution	178,174.7700	9	19,797.19600	246.424	2.10
RESIDUE	3,615.2031	45	80.33785		
TOTAL (CORR)	1,132,096.4000	63			

Appendix 27b

Multiple range analysis for data presented in table 7 on the effect of methanol extract of indicated plants on sclerotia production by S. rolfsii.

Plant species		Count Averages	Homogenous Groups	
A. repens	16	133.0000	A	
S. acuta	16	282.6250		B
B. diffusa	16	286.3125		B
0. corniculata	16	398.8750		C

Figures with different letters are significantly different

Appendix 28a

Analysis of variance of data presented in table 7 showing the effect of methanol extracts on the sizes of sclerotia formed.

Source of variation	Sum of Squares	d.f	Mean Square	Cal.F-ratio	Table F-ratio
MAIN EFFECTS	2295.6020	9	255.06689	20.223	
Species	326.3441	3	108.78137	8.625	2.85
Dilution	1962.6071	3	654.20236	51.869	2.85
Replicates	6.6508	3	2.21694	0.176	
2-FACTOR INTERACTION	834.91715	9	92.768572	7.355	2.10
Species and Dilution	834.91715	9	92.768572	7.355	2.10
RESIDUAL	567.56125	45	12.612472		
TOTAL (CORR)	3698.0804	63			

Appendix 28b

Multiple range analysis for the data presented in table 7 showing the effect of methanol extracts of the indicated plants on the sizes of sclerotia produced by S. rolfsii.

Plant species	Count	Average	Homogenous Groups	
0. corniculata	16	73.2881250	A	
A. repens	16	75.125000	A	B
S. acuta	16	77.218125	A	B
B. diffusa	16	79.405000		B

Figures with the same letters are not significantly different.

Appendix 29a

Analysis of Variance table showing the effect of the alcohol extract of A. indica on the vegetative growth ofj3. rolfsii and Helminthosporium sp. on agar.

Source of variation	Sum of Squares	d.f.	Mean square	Cal.F—ratio	F—ratio (Table)
MAIN EFFECTS	1,110.71880	7	158.67411	28.749	
fungi	552.78150	1	552.78125	100.153	4.32
dilution	538.59380	3	179.53125	32.528	3.07
replicates	19.34380	3	6.44792	1.168	3.07
2-FACTOR INTERACTION	577.59375	3	192.53125	34.883	
fungi and dilution	577.59375	3	192.53125	34.883	3.07
RESIDUE	1 15.90625	21	5.-51935		
TOTAL (CORR>)	1,804.21880	31			

Appendix 29b

Multiple range analysis showing the effect of methanol extract of A. indica on fungal growth.

Fungus	Count	Average	Homogenous Group
Helminthosporium sp.	16	56.6875	A
S. rolfsii	' 16	65.0000	B

Figures with different letters are significantly different.

Appendix 30a

Analysis of Variance table showing the effect of the aqueous extract of Q, anisata on the vegetative growth of S. rolfsii and Helminthosporium sp. on agar.

Source of variation	Sum of Squares	d.f.	Mean square	Cal F-ratio	F—ratio (Table)
MAIN EFFECTS	2613.75	7	373.39286	136.965	
fungi	2.0000	1	2.0000	0.734	4.32
dilution	2605.0000	3	868.33333	318.515	3.07
replicates	6.7500	3	2.25000	0.825	3.07
2-FACTOR INTERACTION	1293.0000	3	431.00000	158.096	
fungi and dilution	1293.0000	3	431.00000	158.096	3.07
RESIDUE	527.250000	21	2.7261905		
TOTAL (CORK)	3964.0000	31			

Appendix 30b

Multiple range analysis showing the effect of aqueous extract of C. anisata on fungal growth.

Fungus	Count	Average	Homogenous Group
Helminthosporium sp.	16	60.5	A
S. rolfsii	16	60.1.	A

Figures with the same letters are not significantly different.

Appendix 3 1a

Analysis of Variance table showing the effect of the methanol extract of C. anisata on the vegetative growth of S. rolfsii and Helminthosporium sp. on agar.

Source of variation	Sum, of Squares d.f. Mean square		Cal F-ratio F-ratio (Table)
MAIN EFFECTS	x 12894.469	7 1842.0670	N 648.438
^ fungi	47.531	1 47.53>3	16.732 4.32
dilution	12842.344	3 4280.7812	1000.000 3.07
replicates	1876.0938	3 1.5312	0.539 3.07
2-FACTOR INTERACTION	1876.0938	3 625.36458	220.139
fungi and dilution	1876.0938	3 625.36458	220.139 3.07
RESIDUE	59.656250	21 2.8407738	
TOTAL (CORR)	14830.219	31	

Appendix 31b

Multiple ranae analysis showing the effect of methanol extract of C. anisata on fungal growth.

Fungus	Count	Average Homogenous Group	
HelminthosDorium SD.	16	32.9375	A
S. rolfsii	16	35.3750-	B

Figures with different letters are significantly different.

Appendix 32a

Analysis of Variance table showing the effect of aqueous extract of Z. xanthoxyloides on the vegetative growth of S. rolfsii and Helminthosporium sp. on agar.

Source of variation	Sum of Squares	d.f.	Mean square	Cal F-ratio	F-ratio (Table)
MAIN EFFECTS	1465.375	7	209.3393	28.113	
fungi	1275.1250	1	1275.1250	171.240	4.32
dilution	177.6250	3	59.2083	7.951	3.07
replicates	12.6250	3	4.2083	0.565	
2-FACTOR INTERACTION	193.12500	3	64.375000	8.645	3.07
fungi and dilution	193.12500	3	64.375000	8.645	3.07
RESIDUE	156.37500	21	7.4464286		
TOTAL (CORR)	1814.8750	31			

Appendix 32b

Multiple range analysis showing the effect of aqueous extract of Z. xanthoxyloides on funaal arowth.

Fungus	Count	Average	Homogenous Group
S. rolfsii	16	56.875	A
Helminthosporium sp.	16	69.500	B

Figures with different letters are significantly different.

Appendix 33a

Analysis of Variance table showing the effect of the alcohol extract of Z. xanthoxyloides on the vegetative growth of S. rolfsii and Helminthosporium sp. on agar.

Source of variation	Sum of Squares	d.f.	Mean square	Cal.F-ratio	F-ratio (Table)
MAIN EFFECTS	10,771.71900	7	1,538.8 1700	1 15.039	
fungi	10,188.78100	1	10,188.78100	761.693	4.32
dilution	573.09400	3	191.03100	14.281	3.07
replicates	9.84400	3	3.28100	0.245	
2-FACTOR INTERACTION	124.84375	3	41.61458	3.11 1	3.07
fungi and dilution	124.84375	3	41.61458	3.111	3.07
RESIDUE	280.90625	21	13.37649		
TOTAL (CORR)	1 1,177.46900	31			

Appendix 33b

Multiple range analysis showing the effect of methanol extract of Z. xanthoxyloides on fungal growth.

Fungus	Count	Average	HomogenousnGroup
Helminthosporium sp. 16		48.9375	A
S. rolfsii	16	84.6250	B

Figures with different letters are significantly different.

Appendix 34a

Multiple range analysis of the effect of methanol extract of A. indica on the vegetative growth of indicated fungi. (Note differences between Scheff Averages for both fungi).

Fungus	Count	Average	Homogeneous Group
HelminthosDorium sd.	16	57.50000	A
S. rolfsii	16	153.37500	B

Appendix 34b

Multiple range analysis ofthe effect of methanol extract of C.anisata on the vegetative growth of indicated fungi in. (Note differences between Scheff Averages for both fungi).

Fungus	Count	Average	Homogeneous Group
HelminthosDorium SD.	16	26.81250	A
S. **rolfsii**	16	102.50000	B

Appendix 34c

Multiple range analysis of the effect on methanol extract of Z. xanthoxyloides on the vegetative growth of indicated fungi. (Note differences between Scheff Averages for both fungi).

Fungus	Count	Average	Homogeneous Group
Helminthosporium sp.	16	75.00000	A
S. rolfsii	16	154.37500	B

Figures with different letters are significantly different.

Appendix 35a

Analysis of variance of dimension of sclerotia formed on medium amended with the aqueous extracts of plants shown in table 10.

Source of variation	Sum of Squares	d.f	Mean Square	Cal.F—ratio	Table F—ratio
MAIN EFFECTS	2884.5033	8	360.56291	24.234	
Species	207.9146	2	103.95731	6.987	3.30
Dilution	2598.6602	3	866.22067	58.220	2.90
Replicates	77.9285	3	25.97615	1.756	
2-FACTOR INTERACTION	917.86058	6	152.97676	10.282	2.20
Species and Dilution	917.86058	6	152.97676	10.282	2.20
RESIDUAL	490.98450	33	14.878318		
TOTAL (CORR)	4293.3484	47			

Appendix 35b

Multiple range analysis for the dimensions of sclerotia formed on media amended with aqueous extract of species shown in table 10.

Plant species	Count	Average	Homogenous Groups
Z xanthoxyloides	16	72.4200	A
C. anisata	16	75.91625	A
A. indica	16	77.38125	A

Figures with the same letters are not significantly different.

Appendix 36a

Analysis of variance of the dimensions of sclerotia formed on media amended with the methanol extract of plants shown in table 10.

Source of variation	Sum of Squares	d.f	Mean Square	Cal.F-rafio	Table F-ratio
MAIN EFFECTS	2321.3004	8	290.16255	10.209	
Species	1239.6914	2	619.84570	21.809	3.30
Dilution	1052.8693	3	350.95643	12.384	2.90
Replicates	28.7397	3	9.57990	0.337	
2-FACTOR INTERACTION	1709.8090	6	284.96816	10.026	2.20
Species and Dilution	1709.8090	6	284.96816	10.026	2.20
RESIDUAL	937.9199	33	28.421818		
TOTAL (CORR)	4969.0293	47			

Appendix 36b

Multiple range analysis for the dimension of sclerotia formed on media amended with methanol extracts of species shown in table 10.

Plant species	Count	Average	Homogeneous	Groups
A. indica	16	71.217500	A	
Z. xanthoxyloides	16	75.660675	A	
C. anisota	16	86.187500		B

Figures with the same letters are not significantly different.

Appendix 37a

Analysis of variance for the number of sclerotia formed In the aqueous extract of plants shown In table 10.

Source of variation	Sum of Squares	d.f	Mean Square	Cal.F—ratio	Table F-ratic
MAIN EFFECTS	388258.33	8	4853.229	362.578	
Species	71075.17	2	35537.58	265.496	3.30
Dilution	316588.33	3	105529.44	788.395	2.90
Replicates	594.83	3	198.28	1.481	
2-FACTOR INTERACTION	32540.167	6	5423.3611	40.517	2.20
Species and Dilution	32540.167	6	5423.3611	40.517	2.20
RESIDUAL	4417.1667	33	133.85354		
TOTAL (CORR)	425215.67	47			

Appendix 37b

Multiple range analysis for the number of sclerotia formed on media amended with aqueous extract of species shown in table 10.

Plant species	**Count**	**Average**	**Homogenous Groups**		
A. indica	16	203.750	A		
C. anisata	16	241.125		B	
Z. xanthoxyloides	16	297.375			C

Figures with different letters are significantly different.

Appendix 38a

Analysis of variance table for the number of sclerotia formed by the methanol extracts of plants shown in table 10.

Source of variation	Sum of squares	d.f	Mean square	Cal.F-ratio	Table F-ratio
Main Effect	749,753.8	8	93,719.23000	403.119	
Species	263,890.7	2	131,945.33000	567.655	3.3
Dilution	485,843.7	3	161,947.89000	696.732	2.9
Replicates	19.5	3	6.50000	0.028	
2-Factor Interaction	291,897.3	6	48,649.55600	209.300	2.40
Species and Dilution	291,897.3	6	48,649.55600	209.300	2.40
Residual	7,670.5	33	232.43939		
Total(corr)	1,049,321.7	47			

Appendix 38b

Multiple range analysis for the number of scelerotia formed by media amended with methanol extracts of species shown in table 10.

Plant species	Count	Average	Homogeneous Groups		
C. anisata	16	120.75	A		
Z. xanthoxyloides	16	198.25		B	
A. 'indica	16	301.75			C

Figures with different letters are significantly different.

Appendix 39

Analysis of Variance of data obtained in Table 17 showing the effect of the aqueous extracts of C. roseus and M. Inermis on sclerotia production by S. rolfsii after 14 days at 2B°C.

Source of variation	Sum of Squares	d.f.	Mean square	Cal. F-ratio F-ratio (Table
MAIN EFFECTS	120357.120	7	17193.875	846.840
species	12561.13	1	12561.125	618.666 8.02
dilution	107725.37	3	35908.458	1000.00 4.87
replicates	70.63	3	23.542	1.159
'-FACTOR INTERACTION	101.37500	3	33.791667	1.664 4.87
species and dilution	101.37500	3	33.791667	1.664 4.87
RESIDUE	426.37500	21	20.303571	
TOTAL (CORR)	120884.88	31		

Appendix 40

Analysis of variance of data obtained in Table 17 showing the effect of the methanol extracts of C. roseus and *M.* Inermis on sclerotia production by S. rolfsii after 14 days at 28°C.

Source of variation	Sum of Squares	d.f.	Mean square Cal.	F—ratio	F—ratio (Table
MAIN EFFECTS	400629.47	7	57232.78	922.644	
species	255076.53	1	255076.53	1000.00	8.02
dilution	145331.84	3	48443.95	780.960	4.87
replicates	221.09	3	73.70	1.188	
-FACTOR INTERACTION	10477.094 '	3	3492.3646	56.300	4.87
species and dilution	10477.094	3	3492.3646	56.300	4.87
RESIDUE	1302.6563	21	62.031250		
TOTAL (CORR)	412409.22	31			

Appendix 4 1

Radial growth of the mycelium of S. rolfsii buried in undiluted methanol leaf extract of Cassia alata for the indicated period before plating on agar medium at28°Cfor14days.
(Data provided Fig. 14).

Period of immersion (h)	Mean d	iameter of	cdlony	(mm)	after (days)	No. of sclerotia formed after 8 days
	1	2	3	4	5	
1/2	-	24.75	42.50	64.5	90.00	72 ±2.74
1	-	20.15	39.80	55.5	86.75	65 ±2.38
6	-	20.20	30.67	53.0	75.00	61 + 0.82
12	-	20.50	30.12	53.0	67.65	56 ± 2.48
18	-	15.60	30.00	48.5	72.00	53 + 1.68
24	-	13.00	26.00	48.0	48.25	52 ± 1.82
48	-	-	-	-	-	-
Control	19.50	45.50	90.00	90.0	90.00	87 13.29

Appendix 42

Radial growth of the mycelium of Helminthosporium sp. buried in undiluted methanol leaf extract of C. alata for the indicated periods before plating on agar medium at 28°C for 14 days (Data provided Fig. 17).

Period of immersion (h)	Mean diameter of colony (mm) after (days)						
	1	2	3	4	5	6	7
1/2	12.00	29.00	38.90	51.50	60.30	73.80	85.00
1	11.70	27.70	39.60	50.50	58.30	71.80	83.00
6	11.50	26.80	38.90	48.30	52.00	66.30	80.25
12	11.00	25.40	38.50	47.00	53.00	64.60	78.50
18	10.75	25.50	36.25	44.80	54.25	63.00	75.00
24	10.00	' 24.50	35.30	43.30	54.50	61.80	70.90
48	9.30	21.50	31.30	38.80	52.30	59.60	66.90
Control	12.30	30.30	44.50	59.30	66.80	77.50	84.30

Appendix 43a

Multiple range analysis showing the effect of incubation period on vegetative growth of the fungi in liquid medium.

Incubation Period (hr.)	Count	Average	Homogenous Group
48	'8 N	30.00	A
24	8	31.50	A B
18	8	33.00	A B
12	8	35.50	A B
6	8	37.50	A B C
1	8	44.25	B C
1/2	8	48.50	C
Control	8	158.50	D

Appendix 43b

Multiple range analysis showing the effect of incubation period on the number of sclerotia formed bv S. rolfsii. in liquid medium.

Incubation Period (hr.)	Count	Average	Homogenous Group
48	4	0.00	A
24	4	52.00	B
18	4	53.00	B C
12	4	56.00	B C
6	4	61.00	B C D
1	4	65.00	C D
1/2	4	72.00	D
Control	4	87.00	E

Figures of the same letters are not significantly different.

Appendix 43c

Multiple range analysis showing the effect of incubation period on vegetative growth of the fungi on solid medium.

Incubation Period (hr.)	Count	Average	Homogenous Group
48	8	33.500	A
24	8	59.500	B
18	8	61.750	B C
12	8	65.750	C D
6	8	66.625	D
1	8	69.250	D
1/2	8	74.750	E
Control	8	87.125	F

Figures of the same letters are not significantly different.

Appendix 43d

Vegetative growth of S. rolfsii buried in undiluted methanol leaf extract of C. alata for the varying periods before incubation in liquid medium at 28°C for 8 days.
(Data provided Fig. 16)

Period of immersion (h)	pH of medium		Mean dry weight of mycelium (mg)	
	Initial	Final	Mean	4 S.E
1/2	3.53	2.56	50	4.56
1	3.53	2.56	46	1.83
6	3.53	2.61	43	2.50
12	3.53	2.72	40	2.04
18	3.53	2.67	36	1.08
24	3.53	2.70	33	1.47
48	3.53	2.71	30	1.47
Control	3.53	1.61	170	4.08

Appendix 43e

Vegetative growth of the mycelium of Helminthosporium sp. at 28°C after being buried in undiluted methanol extract of *Q. alata* for the indicated periods (Data provided Fig. 17)

Period of immersion (h)	pH of medium	N	Mean dry weight of mycelium (mg)	N
1/2	3.53	2.59	47 2.86	
1	3.53	2.52	43 2.50	
6	3.53	2.54 .	33 2.50	
12	3.53	2.60	27 1.22	
18	3.53	2.70	30 0.00	
24	3.53	2.72	30 0.00	
48	3.53	2.72	30 3.54	
Control	3.53	3.80	147 4.36	

Appendix 44

Analysis of variance table showing the vegetative growth of the mycelium of _S. rolfsii and Helminthosporium sp. buried in methanol extracts of C. alata. for varying periods on solid medium.

Source of variation	Sum of Squares	d.f.	Mean square	Cal.F—ratio	F-ratio (Table)
MAIN EFFECTS	/ 24300.312	11	2209.119	678.859	
fungi	N 11183.062	1	11183.062	1000	*.24
incubation	13108.187	7	1872.598 X	575.446	3.07
replicates	9.063	3	3.021	0.928	
2-FACTOR INTERACTION	5462.1875	7	780.31250	239.789	3.07
fungi and incubation	5462.1875	7	78.31250	239.789	3.07
RESIDUAL	146.43750	45	3.2541667		
TOTAL (CORR)	29908.938	63			

Appendix 45

Multiple range analysis showing the vegetative growth of fungi incubated in undiluted methanol extract of_C. alata for varying periods before plating on solid medium.

Fungus	Count	Average	Homogenousn Group
S. rolfsii	32	51.5625	
Helminthosporium, sp.	32	78.0000	

Figures with different letters are significantly different.

Appendix 46

Analysis of variance table showing the vegetative growth of the mycelium of S. rolfsii and Helminthosporium sp. buried in methanol extracts of C. alata. for varying periods in liquid medium.

Source of variation	Sum of Squares	d.f.	Mean square	Cal.F-ratio	F-ratio (Table)
MAIN EFFECTS	106875.06	1 1	9715.915 '	348.814	
fungi	945.56	N 1	945.563	33.947	7.24
incubation	105857.94	7	15122.562	542.919 N	3.07
replicates	71.56	3	23.854	0.856	
2-FACTOR INTERACTION	782.9375	7	111.84821	4.015	3.07
fungi and incubation	782.9375	7	1 11.84821		3.07
RESIDUAL	1253.4375	45	27.854167		
TOTAL (CORR)	108911.44	63			

Appendix 47

Multiple range analysis showing the vegetative growth of fungi incubated in undiluted methanol extract of CL alata for varying periods before innoculating in extract-free liquid medium.

Fungus	Count	Average	HomogenousnGroup
Helminthosporium, sp.	32	48.2500	A
]§. rolfsii	32	55.9375 '	B

Figures with different letters are significantly different.

Appendix 48

Multiple range analysis of data presented in Fig 19

Fungus -	Count	Average	Homogeneous Group
A. niaer	16	55.3750	N A
A. flavus	16	62.5625	B
F. oxvsporium	16	90.0000	C

Appendix 49

Multiple range analysis of data presented in Fig 20

Fungus	Count	Average	Homogeneous Group
A. **niqer**	16	49.250	A
A. **flavus**	16	69.625	B
F. oxysporium	-16	59.500	C

Figures with different letters are significantly different.

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