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# DEGRADATION OF COCOYAM (*XANTHOSOMA SAGITTIFOLIUM L.*) BY THREE ISOLATES OF *SCLEROTIUM ROLFSII* SACC. AND ITS CONTROL.

BY

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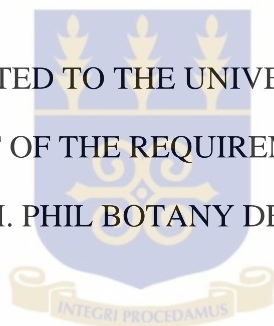
THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF M. PHIL BOTANY DEGREE.




DEPARTMENT OF BOTANY
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# DEDICATION

Dedicated to my two sons Nana Yaw Saka-Yeboah and Kwadwo Ofosu-Saka.

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## ACKNOWLEDGEMENTS

I wish to express my most sincere gratitude to my supervisor, Dr. Ebenezer Owusu for his enthusiastic support, patience, frankness and incisive criticisms and most of all his tolerance and guidance throughout this difficult and demanding period.
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## ABSTRACT

The morphological characteristics of three isolates of *Sclerotium rolfsii* from Aburi (AB), East Legon (EL) and Legon campus (LC) were studied on PDA at an incubation temperature of 28ºC. Radial growth of mycelia and the production of sclerotia were more pronounced in the East Legon (EL) and Legon campus (LC) isolates.

When the red and white cormels of cocoyam (*Xanthosoma sagittifolium*) were inoculated with the various isolates of *Sclerotium rolfsii*, the white cormels were more resistant to infection by the fungus as compared to the red cormels. After cultivating the red and white types of cocoyam on soils inoculated with sclerotia of the three isolates, phenotypic development was more suppressed in the red type of cocoyam than the white type, whilst the yield of cormels was greater in the white type than the red type of cocoyam.

The effects of ethanol and aqueous leaf extracts of plantain, cassava and cocoyam were studied as related to the physiology of the fungus, *Sclerotium rolfsii* on solid and liquid culture media amended with varying dilutions of the extracts. The studies made were in relation to the radial growth and vegetative growth of mycelia as well as sclerotia production. There were clearly differing responses produced by the three isolates of the fungus to the heat-sterilized phytotoxins present in the solid PDA and liquid PDB even though they all showed dose-dependent responses to the active compounds in the leaves. Clearly the ethanol and aqueous extracts of cocoyam proved to be very effective in the control of radial and vegetative growth of mycelia as well as sclerotia production. The Aburi (AB) isolate in both the solid and liquid media proved to be more responsive to the

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phytotoxins with the Legon campus (LC) isolate being the least responsive with regards to radial and vegetative growth of mycelia as well as the production of sclerotia. Higher concentrations of the heat-sterilized ethanol and aqueous leaf extracts proved to be more effective than the least concentrations due to the greater amount of phytotoxins present. The results of the current study have been discussed on the possible effects of the three isolates of *Sclerotium rolfsii* from Aburi (AB), East Legon (EL) and Legon campus (LC) on corm, cormel and pseudostem development of the red and white types of cocoyam as well as the possible use of the various extracts in an integrated control of the fungus and that further studies are recommended to augment these research findings.

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# CHAPTER ONE

## 1.0 INTRODUCTION AND LITERATURE REVIEW

Cocoyam (*Xanthosoma* sp) is a staple root crop that belongs to the Araceae family. It is native of tropical, central and South America and the Carribean (Montaldo, 1991) and different species within the genus *Xanthosoma* have been cultivated and consumed since the pre-coloumbian epoch (Lopez *et al*. (1995)). It is an important crop in the tropical and sub-tropical areas because it provides carbohydrates, proteins, fats and vitamins and cash income for the farmers (Tambong *et al*. 1997). It represents the only member of the aroid which is predominantly used for food.

It reached West Africa by means of traders, missionaries and travellers and soon became popular worldwide in the 16th and 17th centuries (Brown, 2000). Cocoyam was introduced into Asia, the Pacific and North America in the 19th century (Brown, 2000). The predominant species of cocoyam cultivated is *Xanthosoma* sp which is derived from the greek word "xanthos" meaning yellow and "soma" meaning body. This is basically due to the yellowish colour of the corm and cormel pulp which predominates in several species. *Xanthosoma* basically contains 57 species distributed in the tropical and sub-tropical America and Africa with the most predominant being *Xanthosoma sagittifolium* (Giacometi and Leon, 1994). Other species cultivated based on the leaf shape, pigmentation and other vegetative characteristics are *Xanthosomavi olaceum*, *Xanthosoma atrovirens* and *Xanthosoma caraca*. Three types of *Xanthosoma sagittifolium* cultivated in Cameroon

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based on the texture and the colour of the corms and cormels are classified as "white", "red or pink" and "yellow". The ability of the red and white cocoyams to hybridize suggests that they belong to the same species. The colour of the stem, cormels and leaves as well as the shape of the cormels are used for classification into species e.g. *Xanthosoma violaceum* has purple foliage whilst the corm and cormels are purple-grey with reddish eyes and purple or red "flesh". On the other hand *Xanthosoma sagittifolium* has green leaves with the cormels and corms having white, yellow or pink "flesh" and a pale brown "skin". *Xanthosoma sagittifolium* has a globose shaped cormel whereas *Xanthosoma violaceum* has an ovate elliptic cormel

Cocoyam is one of the most important tuber and root crops worldwide with the corms, cormels and leaves serving as important sources of carbohydrates for human nutrition and animal feed as well as cash income for farmers (Baker, K.F. 1987) It is a herbaceous monocotyledonous crop with the growth and development cycle divided into three main periods. During the first two months, the growth is slow. This period starts with the sprouting of shoots and ends with the emergence of cormels. The second stage is characterized by a rapid shoot growth until six to seven months after planting and it is during this period that the plant achieves its maximum leaf area, pseudostem diameter and height. During the third period, the leaves start to wilt and the total dry weight of the plant above ground decreases until harvest. It is a strictly warm weather crop cultivated in the tropical and sub-tropical zone between latitude 30 degrees North and latitude 15 degrees South of the equator. It is not well adapted to waterlogged conditions but even though it is a lowland crop it grows well in upland rainfall regions with well drained soils and a climate

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where rainfall (1400-2000 mm) is well distributed throughout most of the year (Epps *et al*. 1961). It grows vigorously under adverse conditions tolerating a certain amount of shade. The crop survives within a soil pH of 5.5 to 6.5 and temperatures between 29 and 30°C. Temperatures lower than 18°C slow leaf growth and temperatures higher than 35°C increase the foliage but limits corm and cormel formation. Generally growth is favoured when the right temperature is between 14 and 20ºC during which the production of carbohydrates is increased. The sagitate ovate leaves are between 1and 2 cm long and arise directly from the corm with long ribbed petioles. The leaves have a marginal vein and two large basal lobes with variable pigmentation (Purseglove, 1972). Flowering is rare but when it occurs the inflorescence consist of a cylindrical spadix of flowers enclosed in a 12-15 cm spathe. The flowers are unisexual with the female flowers located at the base of the spadix and the male flowers at the top. Between the pistillate flowers and the staminate flowers, sterile flowers are located and that flowering is more prone to occur in the wet regions (Purseglove, 1972). The inflorescence of *Xanthosoma sagittifolium* is protogynous and the female flowers are normally receptive two to four days before pollen is shed. The spadices are seldom fertile and produce few viable seeds.

There has been a steady trend in tropical Agriculture away from the taro, *Colocasia esculenta* cultivation towards increased planting of the *Xanthosoma* sp. Compared with other starchy food crops, cocoyams bring a high yield with a minimum input of labour In 1951, cocoyams were said to be largely replacing taro in the Phillipines because the cormels were larger, more mealy and mucilagenous and the people preferred the taste. In Venezuela, cocoyam production in 1960 was double that of 1937 and totaled 70 000 metric

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tones. Coursey (1968) noted that there was a current rapid shift from taro to cocoyams in Africa which he said was apparently because cocoyam is more suitable for preparing the popular food, a paste formerly made of yams (*Dioscorea* sp). Karikari (1971) said that generally cocoyams are not grown on a large scale, however they are of immense importance in every farm and garden or small holding that this crop is grown. He added that it has an excellent storage quality which makes it a preferred travelling food for the local people. Although it is an important staple food crop in many tropical countries, cocoyam has received low research priority (Geonaga and Hepperly, 1990). In 1975, the National Academy of Sciences classified cocoyam as a neglected crop with economic potential and it is still regarded an under-exploited and insufficiently studied crop (Nguyen and Nguyen, 1987; Giacometti and Leon, 1994; Watanabe, 2002). According to Geonaga and Chardon (1995), yield potential is seldom reached largely due to lack of knowledge concerning diseases, proper management practices and physiological determinants that may limit growth and development. Cocoyam is vegetatively propagated by its corm and cormels and though this type of propagation guarantees the genetic stability of the materials, it however serves as the means by which bacterial, fungal and viral diseases are spread. Dashhen Mosaic Virus (DMV) is associated with all edible aroids and the use of vegetative means of propagation has contributed significantly to the worldwide distribution of the virus (Yam *et al*. 1996). Among the diseases commonly reported to infect cocoyam is the cormel rot which is caused by the pathogen *Sclerotium rolfsii* and some other microorganisms particularly fungi and bacteria (Anyebuno, G.W. 1998).

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The pathogen *Sclerotium rolfsii* Sacc is distributed in tropical and subtropical regions of the world where high temperatures prevail (Arunasri *et al*, 2011). The fungus has a wide host range of 500 species in about 100 families including groundnut, green bean, potato, sweet potato, onion, garden bean, pepper, tomato, cocoyam and water melon (Aycock, 1966). The fungus *S. rolfsii* produces abundant white, fluffy, branched, septate mycelia with clamp connections only on the main hyphae and has a fan-like spread. The mycelia normally form white tufts which later give rise to smooth, hard and dark brown sclerotia which may be spherical or irregular in shape and resembles the mustard seed at maturity (Harrianth Naidu, 2000; Mahan *et al*. 2000 and Arunasri *et al*. 2011). The sclerotial structure was studied by several workers. A sclerotium is known to be made of three layers of an outer rind, a middle cortex and an inner medulla. The size of sclerotia was reported to vary from 0.1mm to 3.0mm (Om Prakash and Singh, 1976; Ansari and Agnihotri, 2001 and Anahosur, 2001). *S. rolfsii* induces a variety of symptoms such as seed rot, seedling blight, collar rot, stem rot and wilt in different host plants. It causes great economic loss in various crops. In groundnut, it caused 25% of seedling mortality in the cultivar JL-24 at Parbhani (Ingale and Mayee, 1986), 30% of crop losses in tomato (Thiribhuvanamala *et al*. 1999) and 40-50% mortality in Crossandra in Chittoor district of Andhra Pradesh (Harianth Naidu, 2000).

The fungus has been recorded in South America in countries such as Argentina, Bermuda Brazil, British Guyana, Colombia, Cuba, Jamaica, Nicaragua, Puerto Rico and Trinidad and Tobago (Erdman, 1961). It is very widespread in warm countries in Africa such as Benin, Democratic Republic of Congo, Egypt, Gambia, Ghana, Malagasy, Nigeria, Sierra

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Leone, South Africa, Togo, Uganda and Zimbabwe (Erdman, 1961). In the Pacific it occurs in countries such as Australia, Indonesia, Java, Malaysia, Phillipines and Sumatra (Erdman, 1961). In the United States of America, the fungus is known to be responsible for the southern blight disease which is thought to be the most widespread disease of vegetables causing serious damage. In Ghana, the fungus causes several serious diseases such as the wilt of tobacco (*Nicotiana tobacum*), bulb rot of onion (*Allium cepa*), wilt of groundnut (*Arachis hypogea*), wilt of potato (*Solanum tuberosum*). Damage caused by *S. rolfsii* in Ghana is estimated to be about 5-30% of harvested crops (FAO, food. 2011)

During infections of natural fields, a clumped or spatial pattern of the inocula of the fungus is observed (Punja *et al*. 1985; Rodriguez-Kabana *et al*. 1974). The same pattern has also been observed in diseased plants (Punja, 1985, Shaw *et al*. 1984). The two main factors affecting the mean population and the frequency of distribution of sclerotia in a field are the sample probe size and the pattern of sampling in the field (Punja *et al*. 1985). The inoculum density of *S. rolfsii* is determined by several methods such as the baiting method, methanol treatment, direct observation and enumeration of sclerotia (Punja *et al*. 1985; Rodriguez-Kabana *et al*. 1974; Madnewesi, J. N. K. 1975). The baiting method involves the use of tissue segments to determine saprophytic growth of the fungus (Avizohar-Hershezon and Shadeed, 1968) as well as the treatment of soil with methanol to stimulate sclerotial germination (Blackman *et al*. Rodriguez-Kabana *et al*. 1980; Shew and Bente, 1984; Shew *et al*. 1984). Sclerotia could be recovered from the soil by wet-sieving (Punja *et al*. 1985) flotation-sieving (Rodriguez-Kabana *et al*. 1974) or elutriation (Shew and Bente, 1984). Factors to be considered in order to establish the correlation between

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inoculums density and disease incidence in a field are the time for the sampling and the location of the samples relative to the host (Punja, 1985). Disease incidence of *S rolfsii* are also affected by environmental factors such as temperature and moisture fluctuations that stimulates germination of sclerotia (Smith, 1972; Punja and Grogan, 1981). The presence of an organic substrate in senescing leaves that promotes mycelia growth could also promote disease severity (Bente and Rodriguez-Kabana, 1979; Beckman and Finch, 1980; MacCarter and Keys, 1984).

Sclerotial infections normally begins at the soil level with the sclerotia being stimulated by drying and remoistening in order to germinate (Punja and Grogan, 1981). Secondary infections spread faster when plants are closer together resulting in the formation of a disease foci (Punja, 1985). Relatively few initial foci can result in extensive damage and failure to produce adequate yield and that in cocoyam where the spread from plant to plant is not extensive due to wider spacing, the yield may not necessarily be indirectly correlated with an incidence in the numbers of disease foci (Rodriguez-Kabana *et al*. 1975). Thus in this case host density and the proximity of roots may be major factors that influences the rate of *S. rolfsii* disease progression (Punja, 1985). Crop rotation with crops susceptible to the pathogen could lead to an increase in the spread of the disease in subsequent years (Rodriguez-Kabana *et al*. 1974; Lockweed, J. L. 1998).

The survival of sclerotia is influenced by factors such as temperature, moisture and the presence of microorganisms around the sclerotia (Punja, 1985). Prolonged temperatures

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above 50ºC are lethal to sclerotia (Porter and Merriman, 1988; Milhail and Acorn, 1984; Yuen and Raabe, 1984). Moist soil contains fewer sclerotia as compared to dry soils (Bandara, 1980; Bente and Rodriguez-Kabana, 1979). High temperatures coupled with high soil moistures are more effective in checking the survival of sclerotia than high temperatures alone. (Bente and Rodriguez-Kabana, 1979) and that sclerotia survives better in surface moist soils than in buried moist soils (Bente and Rodriguez-Kabana, 1979; Javed and Coley-Smith, 1973). The death of buried sclerotia is basically due to increased pressure on the sclerotia by the overlying soil which enhances leakage as well as the presence of antagonistic microorganisms (Punja and Jenkins, 1984)..Mycelia produced by sclerotia are known to survive better in sandy soils than in moist soils (Chattopadyay and Mustafee, 1977). It has also been established that biotic and abiotic factors increase nutrient leakage and activities of soil microorganisms which antagonizes sclerotia are also effective in killing sclerotia faster (Punja, 1985). Most notable antagonistic microorganisms are *Trichoderma* sp (Henis *et al*. 1983) and *Aspergillus sp* (Shigemitsu *et al*. 1978). The antagonistic microorganisms are known to penetrate the rind thus destroying the inner sclerotial tissues by means of the production of enzymes such as β-1,3 glucanase and chitinase (Elad *et al*. 1982; Elad *et al*.1983a; Elad *et al*. 1984) in *Trichoderma* sp.

The management of plant diseases caused by soil-borne pathogens such as *Sclerotium rolfsii* is not feasible with fungicides, mainly because the fungus show strong ability to survive in soil through the formation of dark-brown spherical sclerotia that have strong resistance to both chemical and biological degradation (Punja, 1985; Martini *et al*. 1998)). Again the inappropriate use of agrochemicals especially fungicides has been found to

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possess adverse effect on the ecosystem and possible carcinogenic rise than insecticides and herbicides together (Cameron and Julian, 1984). Moreover resistance by pathogens to fungicides are ineffective (Zhanga and Michailides, 2005). In view of the aforementioned considerations, there may be a need to develop management systems to reduce the dependence on systemic chemicals. The important role that plants play as a source of novel, biologically active compounds is well documented (Recio *et al*. 1989; Sharma and Sharma, 1989; Hostettman and Wolfender, 1997). In recent times, there has been an elevated interest in searching for antimicrobial agents of plant origin as well as in isolating and identifying active components with possible use in integrated crop protection and pest management programmes (Hostettman and Wolfender, 1997; Shivpuri *et al*. 1997). In spite of the substantial information in the literature on the antimicrobial effects of plant extracts on human pathogens (Rabe and Van Stadin, 1989), relatively less information on the efficiency of plant extracts or preparations against plant pathogens and for that matter fungal pathogens is available (Barreto *et al*. 1997).

The quest for plants with medicinal properties continues to receive attention as scientists are in need of plants, particularly of ethnobotanical significance for a complete range of biological activities, which ranges from antibiotic to anticancer (Kaur *et al*. 2011). Several plants and herb species used traditionally have potential antrimicrobial and antiviral properties (Zaika, 1988) and this has raised the hope of scientists about the future of phyto-antimicrobial agents (Das *et al*. 1999). Leaves may be a source of effective potential compounds and may come to be regarded as an inexhaustible source of friendly pesticides having low human and environment toxicity and being easily degradable. Herbaceous

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plants have been used widely in various ways against fungal diseases. Aqueous leaf extract of *Allium sativum*, *Datura alba* and *Withana sativum* inhibited the growth of *Alternaria alternata*, *Alternaria brassicola* and *Myrothecium roridum* (Shahzad and Ghaffer (1988) reported that *Paecilomyces linacimus*was is effective to inhibit growth of sclerotial fungi such as *Macrophomia phaseolina, Rhizoctoniasolani* and *Sclerotium oryzae* causing root rot in many plants.

As part of research directed towards the determination of plants with antimicrobial activity against *S. rolfsii,* the ability of cocoyam leaves, cassava leaves and plantain leaves to suppress the fungus was tested. The ability of the leaf extracts of plants to suppress growth of *S. rolfsii* and sclerotia production is attributed to the presence of inhibitory volatile active ingredients such as alkaloids and phenolic compounds. These volatile compounds occur in two categories namely constitutive compounds which are present in healthy plants and induced compounds which are formed in response to attack by pathogens. Most often these secondary metabolites of plants have their peculiar individual properties and are known not to play any active role in the metabolism of the plant. These constituents often vary from one plant specie to another. Activities of these plant secondary metabolites are termed as "active principles" and the therapeutic value of plants is attributed to this. Analysis of plant extracts leads to the development of new therapeutic agents as well as providing the clue for the exploration and manufacture of some of these compounds synthetically. Plant leaf extracts have been found to be more repressive than extracts from flowers, stems and roots in the control of fungal microorganisms. Extracts from plant families such as Acanthaceae, Amaranthaceae, Amalydiaceae, Anacardiaceae,

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Aspediaceae, Apocynaceae, Banociaceae, Caesalpinaceae, Meliaceae, Oxalidaceae, Papilionaceae, Polypdiaceae, Rutaceae, Sinapterdaceae, Urticaceae and Violaeae have been found to be effective against fungi such as *Aspergillus fumigates, Aaspergillus niger, Sclerotium rolfsii, Fusarium oxysporum, Rhizoctina solani, Phytophthora palmivora and Mucor mucedo* (Chabra *et al*. 1987; Apertorgbor, 1991, Singh *et al*. 1987; Mules, 1987; Boateng, 1986; Saskena and Tripathi, 1986).

The objectives of the present study were set out as follows:

To determine the morphological characteristics of the various isolates of *Sclerotium rolfsii* from Aburi, East Legon and Legon campus.

To study the phenomenon of aversion using the various isolates.

To study the extent of horizontal and vertical rots produced in cormels of red and white cormels of cocoyam inoculated with *Sclerotium rolfsii*.

To study the vegetative parts of the red and white types of cocoyam after inoculation of the soil in which they were cultivated with sclerotia from the three isolates of the fungus.

To study the use of ethanol and aqueous extracts of cassava, cocoyam and plantain to assess the radial diameter of mycelia on PDA, vegetative growth of mycelia in PDB and sclerotial production on PDA for the three isolates of *Sclerotium rolfsii*.

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# CHAPTER TWO

## 2.0 MATERIALS AND GENERAL METHODS

## I MATERIALS

### (a) Source of Pathogen:

The different isolates of the fungus, *Sclerotium rolfsii* Sacc. were obtained from naturally infected cocoyam plants (Plate 1) from three localities in Ghana, identified as *Sclerotium rolfsii* and stored on Potato Dextrose Agar (PDA) slants. Isolate AB was from a cocoyam (*Xanthosoma sagittifolium* farm at Aburi about 25 k from Accra, EL from a cocoyam (*Xanthosoma sagittifolium*) farm at East Legon about 5 km from the University of Ghana campus and LC from a cocoyam (*Xanthosoma sagittifolium*) farm on the University of Ghana campus.

### (b) Source of cocoyam cormels:

The sound (uninfected) red and white cormels of cocoyam (*Xanthosoma sagittifolium*) used in the course of the work were purchased from the Adawso

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market, Eastern Region and transported in sterilized polyethylene bags to the laboratory.

**(c) Source of cocoyam, plantain and cassava leaves:**

Uninfected leaves of cassava (*Manihot esculentum*), cocoyam (*Xanthosoma sagittifolium*) and plantain (*Musa parasidiaca*) were obtained from the same farm in Aburi, Eastern Region and transported in sterilized polyethylene bags to the laboratory.

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**Plate 1:** Photograph of cocoyam plants infected with *Sclerotium rolfsii* ($x^{1}/_{3}$)

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## II METHODS

### (a) Maintenance of stock cultures

Stock cultures of the test fungus were maintained on slants of PDA, incubated at 28°C and sub-cultured every fortnight.

### (b) Preparation of Potato Dextrose Agar (PDA)

Washed and peeled potato (*Solanum tuberosum* L.) tubers were cut into pieces. Two hundred grammes were boiled in five hundred milliliters of distilled water in an aluminium saucepan until softened. The fluid was then collected by straining the slurry with a white muslin cloth. It was allowed to cool and then poured into a one-litre measuring cylinder and was topped up to the one litre mark on the cylinder with distilled water. The liquid was then transferred into a two-litre Erlenmeyer flask. Ten grammes of dextrose and 15g of agar were added to the strained liquid. The mixture was then heated in a microwave (Sharp R-730A) to melt the agar. The preparation was then autoclaved at 1.1kg steam pressure at 121°C for 20 minutes.

### (c) Preparation of Potato Dextrose Broth (PDB)

Washed and peeled potato (*Solanum tuberosum* L.) tubers were cut into pieces. Two hundred grammes were boiled in five hundred milliliters distilled water in an aluminium saucepan until softened. The fluid was then collected by straining the

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slurry with a white muslin cloth. It was allowed to cool and then poured into a one-litre measuring cylinder and was topped up to the one litre mark on the cylinder with distilled water. The supernatant liquid was then transferred into a two-litre Erlenmeyer flask. Ten grammes of dextrose were added to the strained liquid. The mixture was then heated in a microwave (Sharp R-730A) to melt the agar. The preparation was then autoclaved at 1.1kg steam pressure at 121ºC for 20 minutes.

**(d) Solid medium test involving the aqueous (water) and ethanol extracts.**

Heat-sterilized aqueous (water) and ethanol leaf extracts of plantain, cassava and cocoyam prepared were used to amend PDA to obtain solid agar medium with varying dilutions of 1:1, 1:2 and 1:5 v/v . Twenty milliliters of the varying dilutions were poured into amended Petri dishes which were then inoculated with a 3 mm disc of mycelium taken from the margins of an actively growing 3-day old *Sclerotium rolfsii*. There were 24 replicates for each dilution level. Inoculated dishes were incubated at 28ºC and colony diameter readings were taken starting from the third day after inoculation.

**(e) Liquid medium test involving the aqueous and ethanol extracts.**

Potato Dextrose Broth was amended with either ethanol or aqueous leaf extracts of plantain, cassava or cocoyam to obtain varying dilutions of 1:1, 1:2 and 1:5 v/v Twenty milliliters of each of the varying dilutions was poured into 250 ml Erlenmeyer flasks which were plugged with cotton wool and sterilized at 121ºC

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for 15 minutes. Each flask was then inoculated with a 3 mm disc of mycelium taken from the margins of an actively growing 3-day old *Sclerotium rolfsii* of the various isolates and incubated at 28ºC. Each dilution level consisted of 24 replicates. Mycelia were harvested at intervals of 3,5,7,9,11 and 13 days and their dry weights determined.

**(f) Preparation of the aqueous leaf extracts of plantain, cassava and cocoyam**

An amount of 100 g of the fresh leaves of plantain, cassava and cocoyam each, were washed under running tap and then distilled water. They were sun-dried for 10 days and ground using a pestle and a mortar. Hundred millilitres of distilled water was added to each material, stirred vigorously and left to stand for one hour and then filtered using a 4-ply muslin cloth and then filtered through Whatmans paper No. 41 to obtain aqueous extracts of cassava, cocoyam and plantain. The extract was stored at 4ºC in a refrigerator for further studies.

**(g) Preparation of the ethanol leaf extracts of plantain, cassava and cocoyam**

A 100 g of the fresh leaves of plantain, cassava and cocoyam each, were washed under running tap water and then distilled water. They were sun-dried for 10 days and ground to form a paste using a pestle and a mortar. Hundred millilitres of 70% ethanol was added to each paste, stirred vigorously and left to stand for one hour and then filtered using a 4-ply muslin cloth and then filtered through Whatmans paper No. 41 to obtain suspended ethanol extracts of cassava, cocoyam and plantain. The suspension obtained was evaporated to dryness using a Buchi

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Rotary Evaporator (Buchi, Switzerland).

**(h) Incubation**

Inoculated Petri plates were turned upside down in the incubatior. Erlenmeyer flasks were plugged loosely with non-absorbent cotton wool and kept at 25°C in the incubator.

**(i) Oven Dry Weight Method.**

Vegetative growth of the fungus in the liquid media was assessed by harvesting the mycelia at the end of the incubation period. PDB together with mycelia were poured into a Whatman filter paper folded in the form of a funnel and placed within a glass funnel which emptied into a 250 ml Erlenmeyer flask. The apparatus was left for 24 hours for the PDB to drain. The harvested mycelia on the Whatman filter paper washeated in an oven at 75ºC for 24 hours and allowed to cool in a desiccator . It was then reweighed. The filter paper containing the mycelium was dried in an oven and then reweighed after being cooled in a desiccator. The dry weight of the mycelium was then obtained from the difference

**(j) Assessment of growth**

1. Radial growth on agar was assessed by means of measurements by a ruler along two diameters drawn at the bottom of the Petri dish.
2. Growth of the vegetative parts of cocoyam plants were assessed by means of measurements with a string and a ruler.

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3. Diameters of sclerotia and mycelia were assessed by means of a light microscope. using the eyepiece graticule (ocula)
4. Circumference of the petioles were measured with a string and a ruler.
5. Sclerotium production was assessed by allowing the cultures to grow for up to 14 days and counting the number of them formed.

**(k) Chemicals and glassware**

All the chemicals and glassware that were used were of the British Drug House grade. The Glass Petri dishes were used for the solid medium test whilst the 250 ml capacity Erlenmeyer flasks were used for the liquid medium test.

**(l) Inoculation of Petri plates containing microbiological media with fungal isolates.**

During preparation of Petri plates, a pinch of Streptomycin sulphate powder was put into each Petri plate. Discs of mycelium (3mm) were cut at the edge of an established culture with a flamed cork borer and placed inverted using a flamed inoculating pin at the centre of the plate agar to bring the mycelium in contact with the fresh medium.

**(m) Humidity chambers.**

Desiccators were used as humidity chambers.

**(n) Statistical analysis**

Statistical analysis was carried out on the data using the GenStat 12$^{th}$ edition

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software.

The data was quoted as statistically significant at 5% level of significance.

**(o) Experimental Precautions**

1. All glassware used i.e. Petri dishes, Erlenmeyer flasks and test tubes were thoroughly cleaned before use. The Petri dishes and the Erlenmeyer flasks were sterilized in an electrically heated oven (Gallenkamp Oven 300 plus series) for 16 hours before use.
2. Other glassware such as measuring cylinders and beakers as well as media used such as PDA and PDB were sterilized by autoclaving at 121ºC for 30 minutes at a pressure of 1.05 kg/cm. The ethanol and aqueous (water) leaf extracts were sterilized by autoclaving at 121ºC for 15 minutes at a pressure of 1.05 kg/cm.
3. Three millimeter cork borers as well as inoculating needles were dipped in 70% ethanol and flamed before use.
4. The laminar flow chamber in the inoculating room was thoroughly cleaned with dettol and switched on for 30 minutes before use.
5. Antibiotics were added to the PDA and PDB cultures in order to suppress bacterial growth.
6. The filter paper used for harvesting the mycelia was reasonably of the same size and heated in an oven at 75ºC for 24 hours before use.
7. The filter paper and the harvested mycelia were reasonably kept in desiccators all the time to prevent absorption of moisture from the atmosphere.

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8. The polyethylene bags used for the transport of the cormels were sterilized before use with 70% ethanol.
9. The surface of the cormels were washed in tap water and sterilized with 70% ethanol before use.
10. The cormels of the red and white cocoyam were uninfected and approximately of same size.

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# CHAPTER THREE

## 3.0 EXPERIMENTAL PROCEDURE

### EXPERIMENT 1

### STUDY OF THE MORPHOLOGICAL CHARACTERISTICS OF *SCLEROTIUM ROLFSII* ISOLATES FROM ABURI (AB), EAST LEGON (EL) AND LEGON CAMPUS (LC).

The various isolates comprising AB, EL and LC were sub-cultured on PDA. Three millimeters discs cut by means of a 3 mm cork borer were picked from margins of an actively growing mycelia of each isolate and inoculated separately at the center of the Petri dish. Streptomycin sulphate was added to control bacterial growth. There were 24 replicates for each isolate. The cultures were then incubated at 28ºC for 3 days and the radial growth of the mycelium was measured along two diameters drawn at the bottom of the Petri dish from the 3$^{rd}$ day to the 9$^{th}$ day of incubation. For each day, from the 3$^{rd}$ day to the 9$^{th}$ day, radial growth of four cultures of each isolate were determined and their means calculated. The various cultures were then allowed to develop for 14 days, after which the diameter of sclerotia were estimated with the aid of a light microscope.. The total number of sclerotia on each Petri plate was counted and the average number of sclerotia per disc calculated. The colour of sclerotia was also monitored throughout the incubation period by observation with a hand lens.

The results obtained are presented in Figs 1,2,3 and Table 1..

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## EXPERIMENT 2

## STUDY OF THE PHENOMENON OF AVERSION USING ISOLATES FROM LEGON (ABURI (AB) EAST LEGON (EL) AND LEGON CAMPUS (LC).

Mycelial discs (3mm diameter) taken from the edge of an actively growing colony (3-4 day old) of each isolate were placed approximately 25 to 35mm apart on opposite sides of 90mm Petri dishes and incubated at 28ºC. The plates were examined microscopically after days for the presence of an antagonistic or aversion zone in the region of mycelia contact. The cultures were allowed to develop for 14 days after which the sclerotia formed in the border of the lytic zone were counted. The test was repeated at least twice.

The results obtained are presented in Fig. 4 and Plate 2.

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## EXPERIMENT 3

## STUDIES OF ROT PRODUCED AT SIDE AND BOTTOM OF RED AND WHITE CORMELS OF COCOYAM BY THE THREE ISOLATES OF *SCLEROTIUM ROLFSII*.

Medium sized, sound cormels free from wounds were selected for uniformity from a large

collection. The cormels were cleaned, surface-sterilized with 70% ethanol for 5 minutes. Care was taken in handling of the cormels so that they were not wounded. To test the pathogenicity of the isolates, 3mm disc of each isolate was cut from the periphery of actively growing cultures of the fungi. Discs of sterile PDA, 3mm in diameter was used as control. Each isolate was used to inoculate 24 cormels of each of the red and white cormels of cocoyam. The inoculated cormels were kept in humidity chambers for 21 days at 28ºC. At 3-day interval for 21 days, four inoculated cormels of each type of cocoyam were randomly selected and cut at right angles to the holes through which the inocula were introduced. The extent of rot development was followed by measuring the radius of rot along two radii of rotted area at right angle to each other and their average recorded. The experiment was carried out three times. The process was repeated with tissues removed from the side of each type of cocoyam.

Data obtained are presented in Figs 5 and 6..

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**EXPERIMENT 4:**

**MORPHOLOGICAL CHARACTERISTICS OF THE WHITE AND RED TYPES OF COCOYAM PLANTS PLANTED IN SCLEROTIUM INFESTED SOIL IN STERILIZED POLYETHYLENE BAGS.**

**I** Red and white corms of cocoyam were raised in polyethylene bags containing loamy soil until the vegetative parts started to develop. Each bag was then inoculated with about 25 sclerotia of same age taken from each isolate and covered with 1mm of soil. There were four replicates of white and red cocoyam plants for each of the isolates.. A control experiment was set-up for both the red and white strains of cocoyam in which the soil was not inoculated with sclerotia. The following morphological characteristics length of leaf lamina, width of leaf lamina, circumference of the pseudostem, length of the petiole and the circumference of the petiole were measured for the red and white types of cocoyam.

Readings were taken from 10 days after inoculation of soil with sclerotia and the results are presented in Figs. 7-16.and Tables 2 and 3.

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**II** The raised corms were flooded with water to soften the soil and uprooted after three months of cultivation. The number of cormels then counted and the dry weights of the corms and above soil vegetative parts were then estimated by the oven dry weight method.

The results are presented in Figs. 17-22 and Tables 4 and 5..

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**EXPERIMENT 5:**

**EFFECT OF ETHANOL LEAF EXTRACT OF PLANTAIN ON THE RADIAL GROWTH OF MYCELIA AND PRODUCTION OF SCLEROTIA BY THE THREE ISOLATES OF *SCLEROTIUM ROLFSII*.**

Potato dextrose agar (PDA) medium was amended with heat-sterilized at 121ºC for 15 minutes ethanol dry leaf extract of plantain to obtain undiluted, 1:1, 1:2 to 1:5 v/v of the dry ethanol leaf extract. In order to obtain undiluted ethanol dry leaf extract of plantain, an equal volume of double strength PDA was mixed with that of double strength ethanol leaf extract. The Petri plates containing the varying dilutions of the ethanol extract were inoculated at the centre with one sclerotium of each of the isolates of *S rolfsii* (AB, EL and LC) and then incubated for 5 days at 28ºC. The radial growth of mycelia for each of the isolates were determined by drawing two lines at the bottom of the Petri dish. Each dilution level consisted of 24 replicates for each dilution level. After 14 days of incubation, the number of sclerotia per each dilution was recorded.

The results obtained are presented in Figs.23 and 24.

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**EXPERIMENT 6:**

**EFFECT OF ETHANOL LEAF EXTRACT OF CASSAVA ON THE RADIAL GROWTH OF MYCELIA AND PRODUCTION OF SCLEROTIA BY THE THREE ISOLATES OF *SCLEROTIUM ROLFSII*.**

The experimental set up was exactly as in Experiment 5, except that ethanol leaf extract of cassava was used.

The results obtained are presented in Figs. 25 and 26.

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**EXPERIMENT 7:**

**EFFECT OF ETHANOL LEAF EXTRACT OF COCOYAM ON THE RADIAL GROWTH OF MYCELIA AND PRODUCTION OF SCLEROTIA BY THE THREE ISOLATES OF *SCLEROTIUM ROLFSII*.**

The experimental set up was exactly as in Experiment 5, except that ethanol leaf extract of cocoyam was used.

The results obtained are presented in Figs. 27 and 28.

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**EXPERIMENT 8:**

**EFFECT OF AQUEOUS LEAF EXTRACT OF PLANTAIN ON THE RADIAL GROWTH OF MYCELIA AND PRODUCTION OF SCLEROTIA BY THE THREE ISOLATES OF *SCLEROTIUM ROLFSII*.**

Potato dextrose agar (PDA) medium was amended with heat-sterilized at 121ºC for 15 minutes aqueous dry leaf extract of plantain to obtain undiluted, 1:1, 1:2 to 1:5 v/v of the dry aqueous leaf extract. In order to obtain undiluted aqueous dry leaf extract of plantain, an equal volume of double strength PDA was mixed with that of double strength aqueous leaf extract. The Petri plates containing the varying dilutions of the aqueous extract were inoculated at the centre with one sclerotium of each of the isolates of *S rolfsii* (AB, EL and LC) and then incubated for 5 days at 28ºC. The radial growth of mycelia for each of the isolates were determined by drawing two lines at the bottom of the Petri dish. Each dilution level consisted of 24 replicates for each dilution level. After 14 days of incubation, the number of sclerotia per each dilution was recorded.

The results obtained are presented in Figs.29 and 30.

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**EXPERIMENT 9:**

**EFFECT OF AQUEOUS LEAF EXTRACT OF CASSAVA ON THE RADIAL GROWTH OF MYCELIA AND PRODUCTION OF SCLEROTIA BY THE THREE ISOLATES OF *SCLEROTIUM ROLFSII*.**

The experimental set-up in Experiment 8 was repeated but this time aqueous leaf extract of cassava was used.

The results obtained are presented in Figs. 31 and 32.

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**EXPERIMENT 10:**

**EFFECT OF AQUEOUS LEAF EXTRACT OF COCOYAM ON THE RADIAL GROWTH OF MYCELIA AND PRODUCTION OF SCLEROTIA BY THE THREE ISOLATES OF *SCLEROTIUM ROLFSII*.**

The experimental set-up in Experiment 8 was repeated but this time aqueous leaf extract of cocoyam was used.

The results obtained are presented in Figs. 33 and 34.

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**EXPERIMENT 11:**

**STUDIES ON EFFECT OF HEAT-STERILIZED ETHANOL LEAF EXTRACT OF PLANTAIN ON VEGETATIVE GROWTH OF THE THREE ISOLATES OF *SCLEROTIUM ROLFSII***

In Experiments 5-10, both the ethanol and aqueous extracts depressed radial growth of the three isolates of *S. rolfsii*. In this experiment, the leaf extracts were tested for their effects on dry matter accumulation in the three isolates of the test fungus in Erlenmeyer flasks.

Potato Dextrose Broth (PDB) was amended to obtain varying dilutions 1:1 to 1:5 v/v of heat-sterilized ethanol leaf extract of plantain. Erlenmeyer flasks (250 ml capacity) containing 30 ml of the appropriate dilution of the extract were each inoculated with one 3 mm disc of mycelium of the three isolates (AB, EL and LC) of *S rolfsii* taken from the margins of an actively growing mycelia of the various isolates and incubated at 28ºC. There were 24 replicates for each dilution level for each isolate and at 2-day interval, four of the flasks were taken and the vegetative growth of the mycelia assessed by means of the oven dry weight method (Materials and General Methods).

The results are presented in Fig. 33.

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**EXPERIMENT 12:**

**STUDIES ON EFFECT OF HEAT-STERILIZED ETHANOL LEAF EXTRACT OF CASSAVA ON VEGETATIVE GROWTH OF THE THREE ISOLATES OF *SCLEROTIUM ROLFSII***

The Experiment in 11 was repeated but this time ethanol leaf extract of cassava was used to replace plantain.

The results obtained are presented in Fig. 36.

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**EXPERIMENT 13:**

**STUDIES ON EFFECT OF HEAT-STERILIZED ETHANOL LEAF EXTRACT OF COCOYAM ON VEGETATIVE GROWTH OF THE THREE ISOLATES OF *SCLEROTIUM ROLFSII***

The Experiment in 11 was repeated but this time, ethanol leaf extracts of cocoyam was used in place of plantain.

The results obtained are presented in Fig. 37 and Plate 4.

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## EXPERIMENT 14:

## STUDIES ON EFFECT OF HEAT-STERILIZED AQUEOUS LEAF EXTRACT OF PLANTAIN ON VEGETATIVE GROWTH OF THE THREE ISOLATES OF *SCLEROTIUM ROLFSII*

The Experiment in 11 was repeated but this time with aqueous leaf extract of plantain instead of the ethanol extract.

The results obtained are presented in Fig. 38.

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**EXPERIMENT 15:**

**STUDIES ON EFFECT OF HEAT-STERILIZED AQUEOUS LEAF EXTRACT OF CASSAVA ON VEGETATIVE GROWTH OF THE THREE ISOLATES OF *SCLEROTIUM ROLFSII***

The Experiment in 11 was repeated but this time with aqueous leaf extract of cassava.

The results obtained are presented in Fig. 39..

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**EXPERIMENT 16:**

**STUDIES ON EFFECT OF HEAT-STERILIZED AQUEOUS LEAF EXTRACT OF COCOYAM ON VEGETATIVE GROWTH OF THE THREE ISOLATES OF *SCLEROTIUM ROLFSII***

The Experiment in 11 was repeated but this time with aqueous leaf extracts of cocoyam.

The results obtained are presented in Fig. 40..

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# CHAPTER THREE

## 3.0 EXPERIMENTAL RESULTS

### 1: STUDY OF THE MORPHOLOGICAL CHARACTERISTICS OF *SCLEROTIUM ROLFSII* ISOLATES FROM ABURI (AB), EAST LEGON (EL) AND LEGON CAMPUS (LC)

The morphological characteristics of the three isolates of *Sclerotium rolfsii* grown on PDA plates revealed that the radial growth of mycelia varied up to three days.(Fig. 1). However the isolates achieved the maximum growth after six days of incubation, with the entire plates being covered with mycelia. The formation of sclerotia initiated after 96 hours (4 days) of incubation continued till 192 hours (8 days).The LC isolate had the highest mean number of sclerotia (316) followed by the EL, (267) and AB (183) isolate (Fig.2). Initially, the three isolates produced a white, coloured sclerotia which later on transformed into light brown and then dark brown as shown in (Table 1). The sclerotia of the EL isolate were found in zones whereas those of the LC and AB isolate were evenly distributed on the agar plates. The shape of the sclerotia of the isolates were mostly round with the sclerotial diameter of the three isolates ranging from 5.9 to 7.2 µm. The EL isolate had the largest diameter (7.2 µm) followed by the LC isolate (6.3 µm) and the AB isolate 5.9 µm (Fig. 3).

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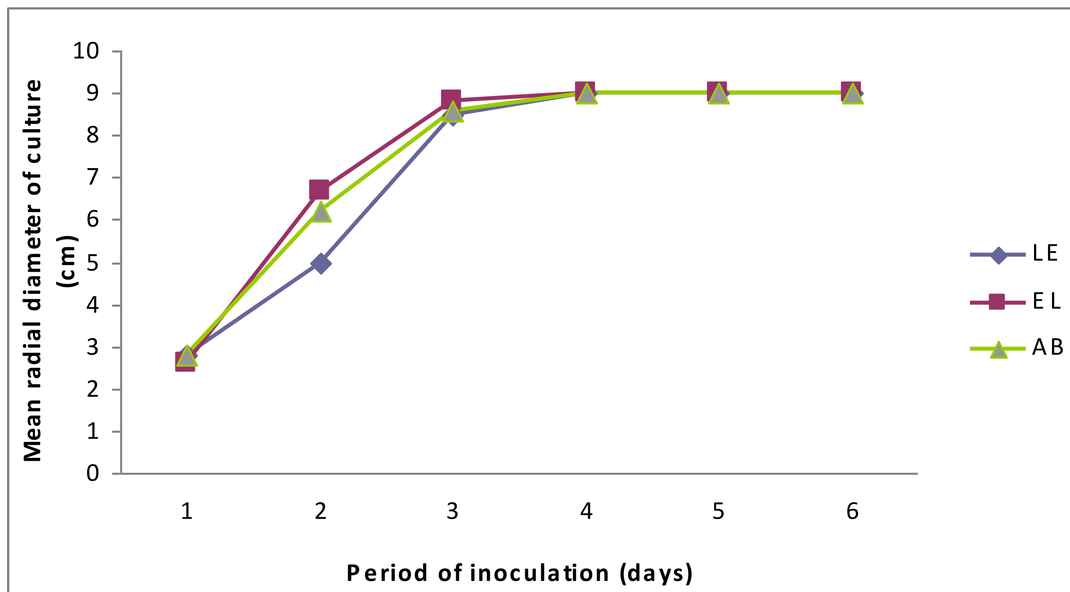


**Fig. 1**. Radial growth of the three isolates of *S. rolfsii* after 8 days of incubation on PDA at 28ºC.

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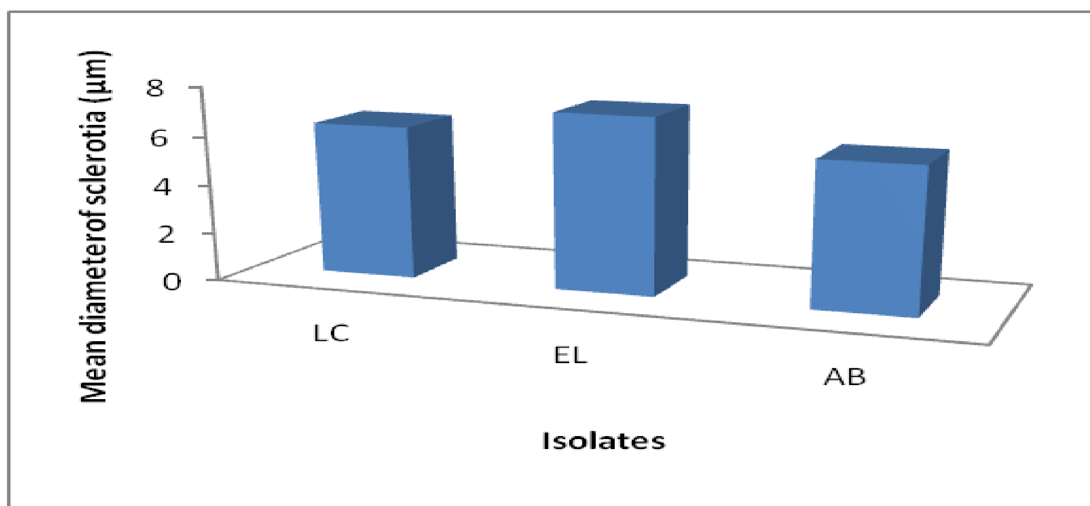


**Fig. 2**. Mean diameter of sclerotia by the three isolates of *S. rolfsii* on PDA plates during 14 days of incubation at 28ºC.

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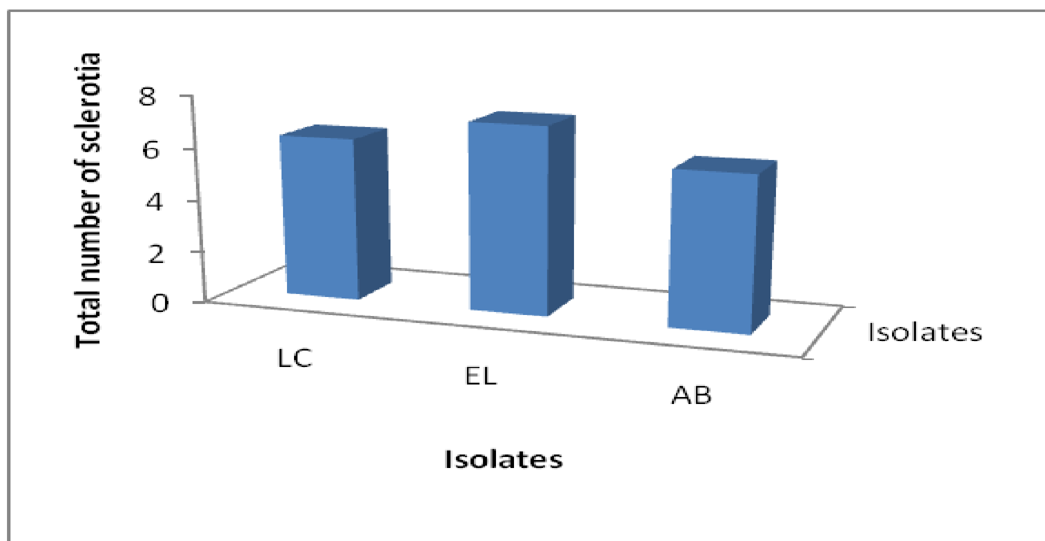


**Fig. 3**. Total number of sclerotia of the three isolates of *S. rolfsii* after 14 days of incubation at 28ºC.

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**TABLE 1 :** Colour formation by sclerotia of the three isolates of *Sclerotium rolfsii* in indicated incubation period at 28ºC.

| Period of incubation | Isolates of *Sclerotium rolfsii* | | |
|---|---|---|---|
| (Days) | Aburi (AB) | East Legon (EL) | Legom Campus (LC) |
| 48 | - | - | - |
| 72 | - | - | - |
| 96 | - | - | - |
| 120 | White | White | White |
| 144 | Light brown | Light brown | Light brown |
| 168 | Dark brown | Dark brown | Dark brown |
| 192 | Dark brown | Dark brown | Dark brown |

- No sclerotia produced.

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## 2: STUDY OF THE PHENOMENON OF AVERSION USING ISOLATES OF *SCLEROTIUM ROLFSII* FROM ABURI (AB), EAST LEGON (EL) AND LEGON CAMPUS (LC).

The three isolates showed antagonistic reactions with each other. In all the antagonistic reactions, sclerotia formed only in the border of the lytic zone of the isolates (Plate 2). The mycelia of the various isolates were distinctly separated from each other by a clear zone referred to as the lytic zone and that sclerotia of the various isolates were formed at the edges that separates the mycelia of the various isolates. Fewer sclerotia produced later on such lytic zones failed to develop to their fullest sizes as those produced at the border of the zones.

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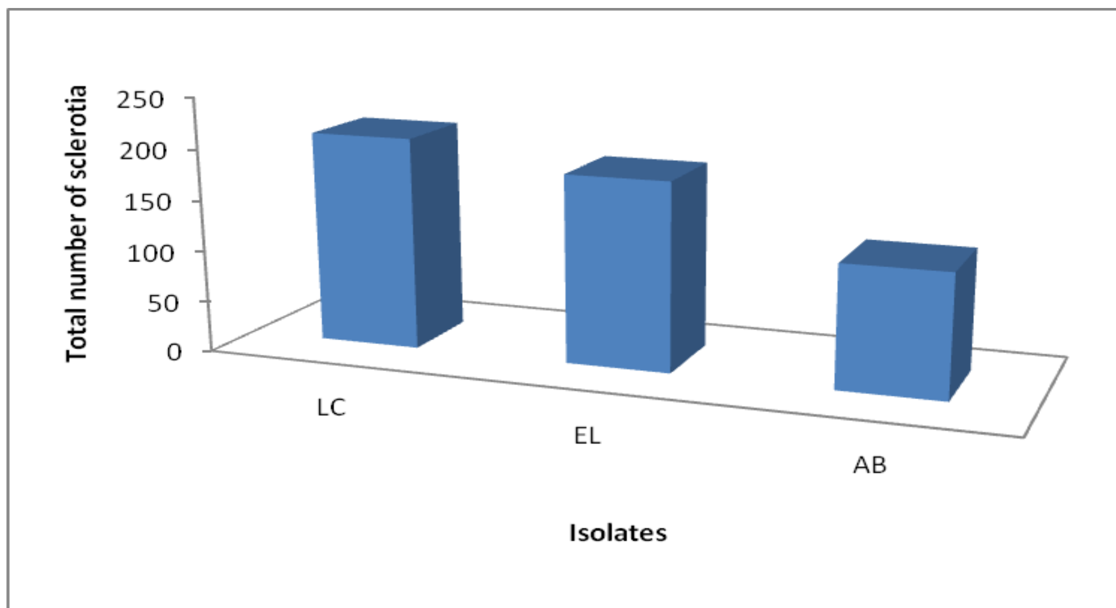


**Fig. 4**. Total number of sclerotia of the three isolates of *Sclerotium rolfsii* after 14days of incubation at 28ºC using the phenomenon of aversion..

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**Plate 2:** Photograph showing mycelia incompatibility reactions between isolates of *Sclerotium rolfsii* ($x^1/_3$)

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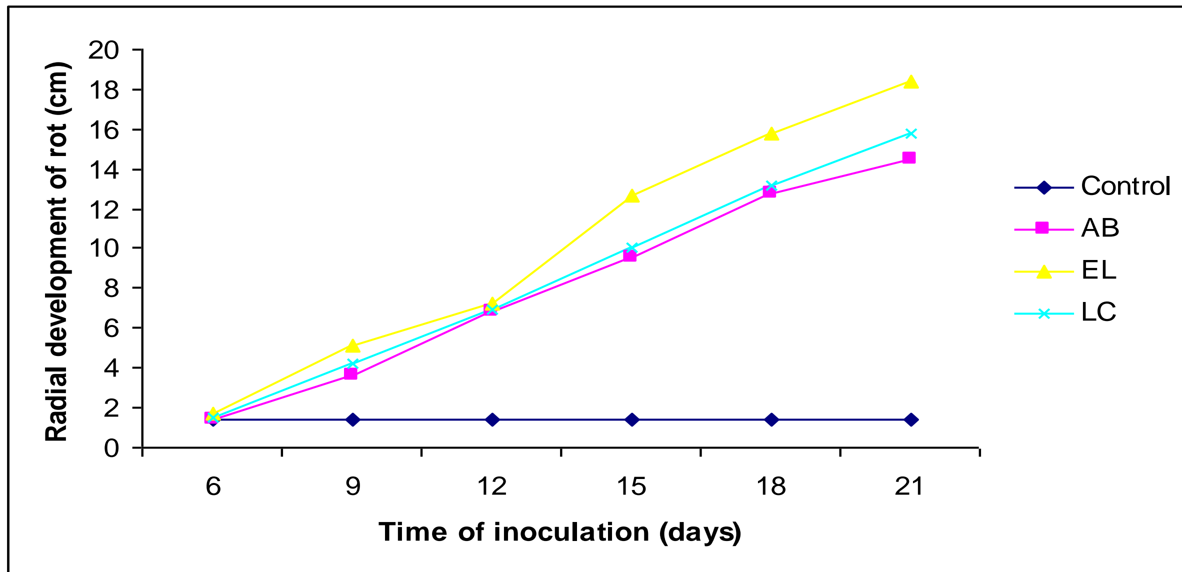
## 3 : STUDIES OF ROT PRODUCED AT SIDE AND BOTTOM OF RED AND WHITE CORMELS OF COCOYAM INOCULATED WITH THE THREE ISOLATES OF *SCLEROTIUM ROLFSII.*

The mean diameter of rot of the vertical (bottom) inoculation of the red type of cormel was greatest in that inoculated with the EL isolate after the maximum period of 21 days of the study. This was followed by the LC and the AB isolates respectively (Fig.5). All the isolates of *Sclerotium rolfsii* produced a gradual increase in rot with increasing number of days. A similar trend was observed in the white cormels, however there was a decline in amount of rot of the white cormels as compared to the red cormels (Fig. 5).

The horizontal or side rot of the white cormels was greatest in the AB isolate of *Sclerotium rolfsii* as compared to the EL) and LC isolates (Fig. 6)., however the differences were very marginal. In the red cormels, the greatest rot was produced by the EL isolate followed by the AB isolate and the LE isolate in that order (Fig. 7). The rots were all of the dry type, with the diseased tissue being firm or cakey. The colour of the cocoyam tissue in the course of the rot changed progressively from cream white to various shades of brown or grey and terminated in dark brown. In some cases, fungal structures in the form of white mycelia cover or brown sclerotia were observed on the surface of the rotten cormels.

**Red**




**White**

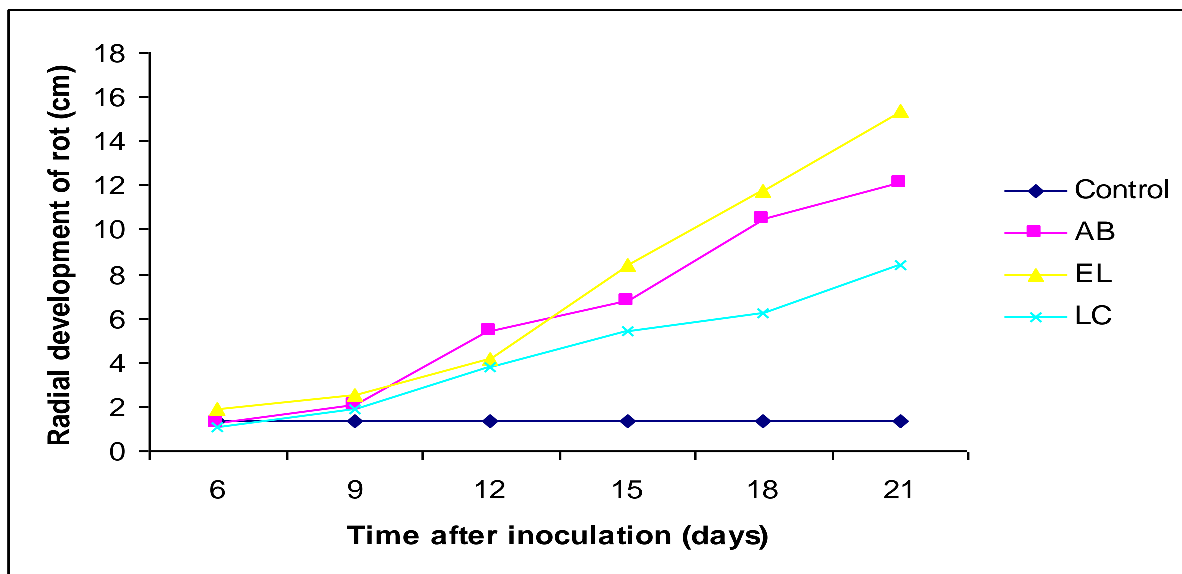



**Fig. 5**. Time for rot development at the bottom of the red cormel and side of the white cormel of cocoyam inoculated with the three isolates of *S. rolfsii* at 28ºC.

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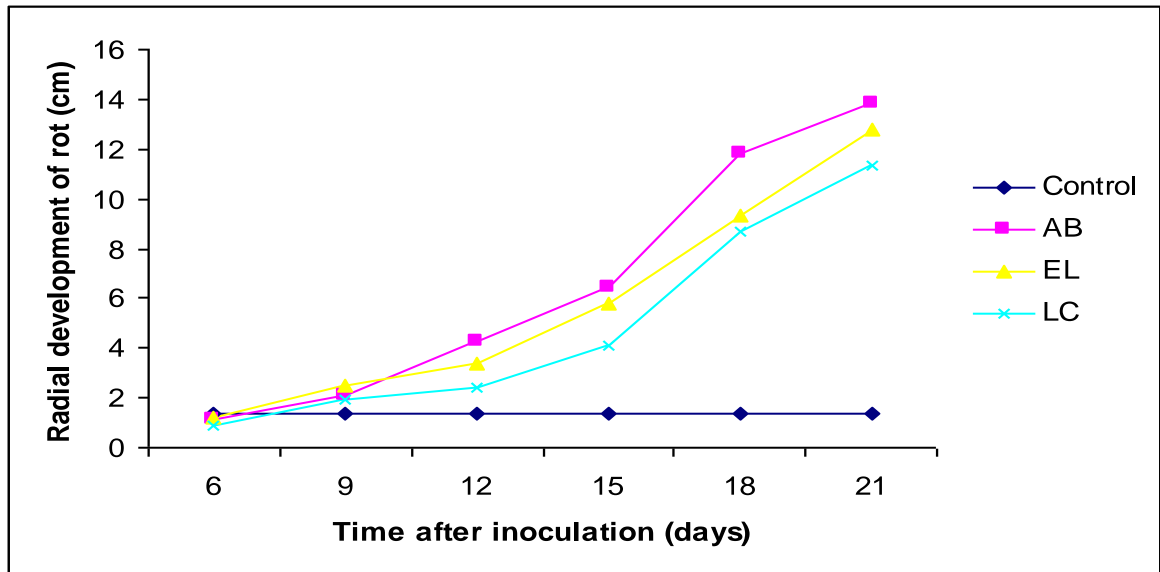
**Red**




**White**

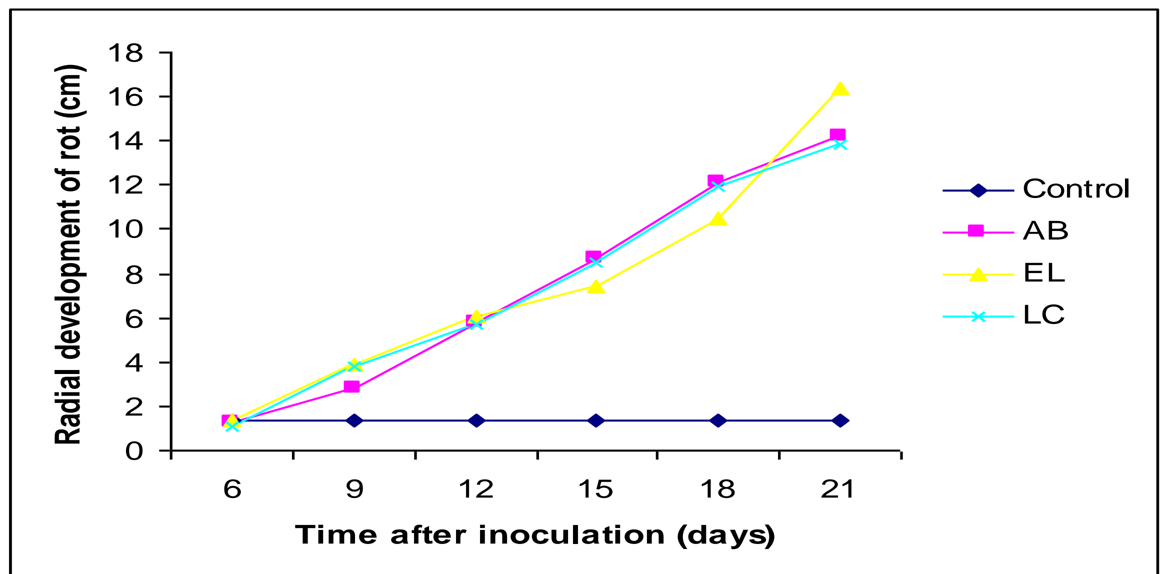



**Fig. 6**. Time for rot development at the bottom of the white cormel and side of the red cormel of cocoyam inoculated with the three isolates of *S. rolfsii* at 28ºC.

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# 4: MORPHOLOGICAL CHARACTERISTICS OF THE WHITE AND RED TYPES OF COCOYAM PLANTS PLANTED IN SCLEROTIA INFECTED SOIL IN STERILIZED POLYETHYLENE BAGS.

The data obtained for the morphological characteristics of the red and white corms plante in soil inoculated with the three isolates of *Sclerotium rolfsii* after the maximum period of study are presented in Figs. 7-22 and Tables 2-5. The data obtained for the following phenotypic characteristics: length of leaf lamina, width of leaf lamina, circumference of pseudostem, length of petiole and circumference of the petiole of the white cocoyam are presented in Figs. 7-11 and that of the red type are presented in Figs 12-16.The yield components viz dry weight of shoot, dry weight of the corm and number of cormels of the two types of cocoyam are presented in Figs. 17-22. The mean length of the leaf lamina of the white and red types of cocoyam increased with increasing number of days (Fig.8). The greatest length (15.8mm) was obtained in the plants potted in bags inoculated with the EL isolate of *Sclerotium rolfsii* for the white type of cocoyam (Fig. 8) and the LC isolate of *Sclerotium rolfsii* for the red type of cocoyam plants. The mean length of leaf lamina of the plants inoculated with the AB isolate of *Sclerotium rolfsii* approximated that of the plants inoculated with sclerotia from the LC isolate for the maximum duration of the study

(Fig. 8).

A similar trend of a gradual increase was obtained in the estimation of the mean width of the leaf lamina for both the red and white types of cocoyam plants inoculated with the

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sclerotia of LC, EL and the AB isolates of *Sclerotium rolfsii*. The maximum mean width (14.0mm) of the leaf lamina was recorded in the black bag inoculated with the sclerotia of the EL isolate for the white strain of cocoyam and sclerotia of the LC (11.2) isolate for the red type of cocoyam.

The mean circumference of the pseudostem for both the red and white cocoyam plants showed marginal increase.. The bag inoculated with sclerotia of the AB isolate produced plants with least mean circumference of petiole for the white strain of cocoyam (Fig.11), while the cocoyam plants raised in bags inoculated with sclerotia of the EL isolate for the red strain of cocoyam produced plants with the least mean circumference of the stem (Table 3) though the differences between the plants inoculated with sclerotia of the various isolates were marginal.

There were marginal differences as regards to the mean length of the petiole in the plants raised in bags inoculated with various isolates of *Sclerotium rolfsii*. The mean length of the petiole for the plants infected with sclerotia from the EL isolate approximated that of the control for the white strain of cocoyam (Fig.10 ), however there were marked differences in results produced for the red type of cocoyam plants in response to infection by the various isolates of *Sclerotium rolfsii* (Fig. 15)

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The mean circumference of the petiole for the control of both the red and white strains of cocoyam were the same for the maximum duration of the study. There was a remarkable variation among the plants infected by sclerotia of the EL isolate as compared to that produced in plants by sclerotia of the AB) and the LC isolates for the white type of cocoyam. For the red type of cocoyam, both the EL and the LC isolates produced a similar mean circumference of the petiole for the maximum duration of the study (Fig. 16)..

Plants cultivated in soil infected with sclerotia of the LC isolate gave the highest estimation of 5.8g mean dry weight of the upper vegetative parts for the white type of cocoyam plants followed by those inoculated with the AB isolate, 4.6g and the EL isolate, 3.6g respectively (Fig 17). For the red type of cocoyam however, the AB isolate produced the highest estimation of the dry weight of the shoot followed by the EL and the LC isolates in that order (Fig. 18).

The repressive effect of *Sclerotium rolfsii* with regards to its influence on the development of the corm was minimal in the LC isolate infected plants followed by the EL and AB isolate infected plants. This was manifest in the estimation of the dry weights of the corms infected with the various isolates after the maximum duration of study for the white type of cocoyam (Fig.19). In sharp contrast for the red strain of cocoyam, the mean dry weights of the corms infected with the various isolates were very minimal and that the effect of the East Legon (EL) isolate was most greatest as compared to the LC and the Aburi AB isolates in that order (Fig.20)..

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The destructive effect of *Sclerotium rolfsii* was manifested in the development of cormels for both the red and white types of cocoyam. For the white type of cocoyam plants infected with both the AB and the EL isolates produced the same mean number of cormels for the entire duration of the study (Fig. 21). The least number of cormels were produced by the LC infected plants isolate . On the contrary, for the red type of cocoyam plants infected with the AB and the LC isolates produced the same mean number of cormels(1) whilst the EL isolate produced the least number of zero cormels. (Fig. 22).

A significant observation made in the estimation of the dry weight and the number of cormels is that the results produced by the plants infected with the various isolates were sharply lower than that of the control for both the red and white strains of cocoyam.

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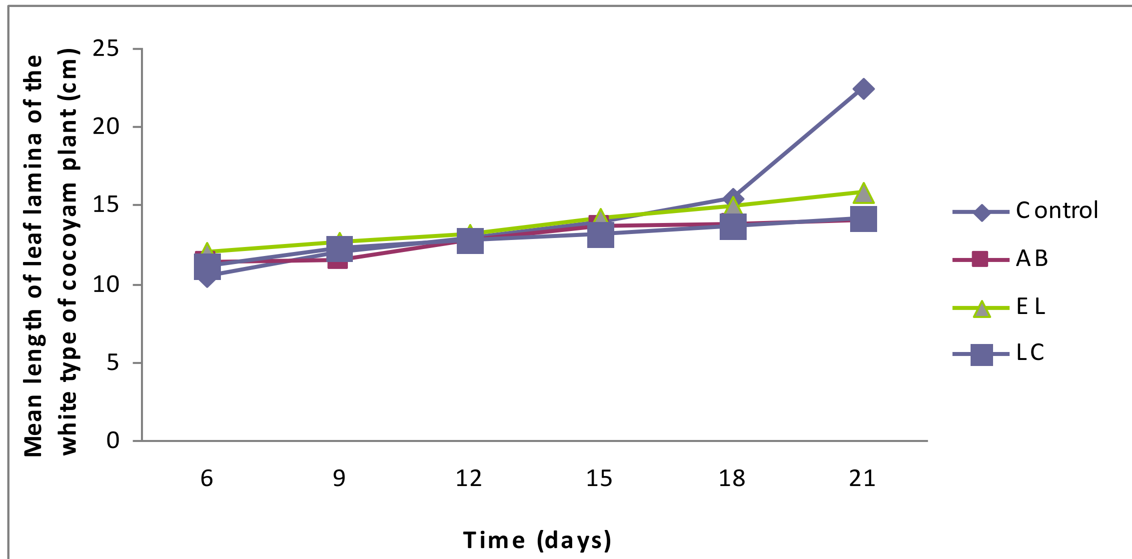


**Fig. 7**. Effect of the three isolates of *S. rolfsii* on the length of leaf lamina of white type of cocoyam plant.

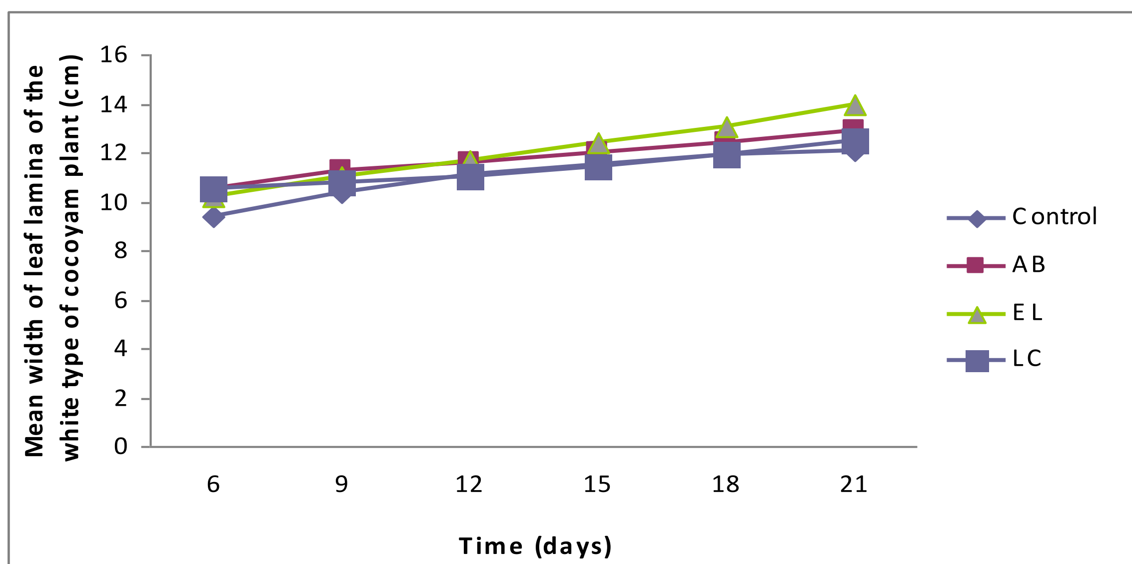


**Fig. 8**. Effect of the three isolates of *S rolfsii* on the width of leaf lamina of white type of cocoyam

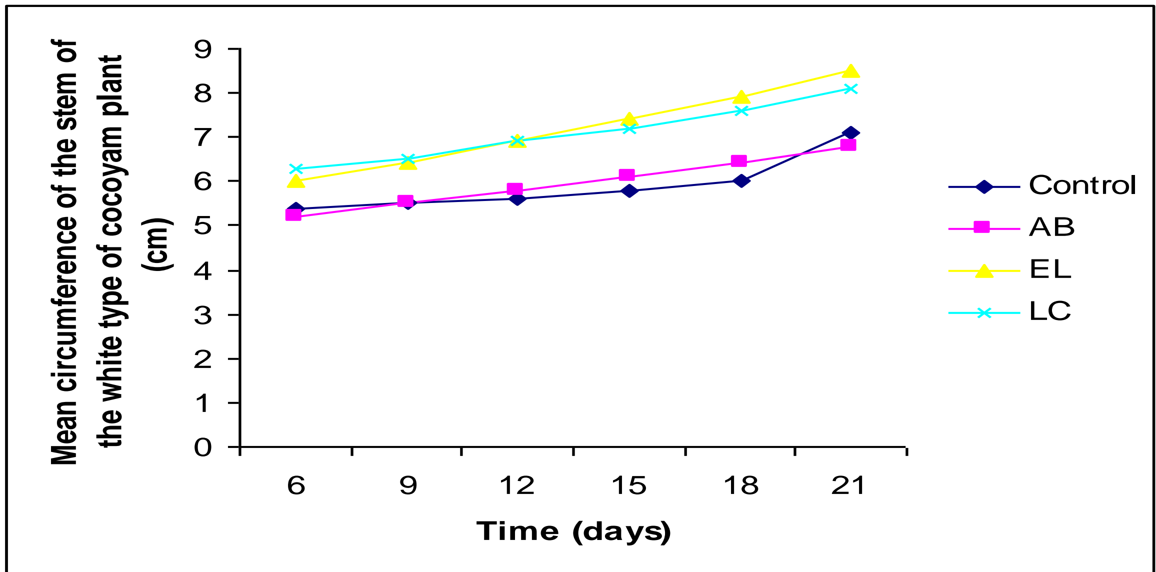


**Fig. 9**. Effect of the three isolates of *S. rolfsii* on the circumference of the stem of the white type of cocoyam plant.

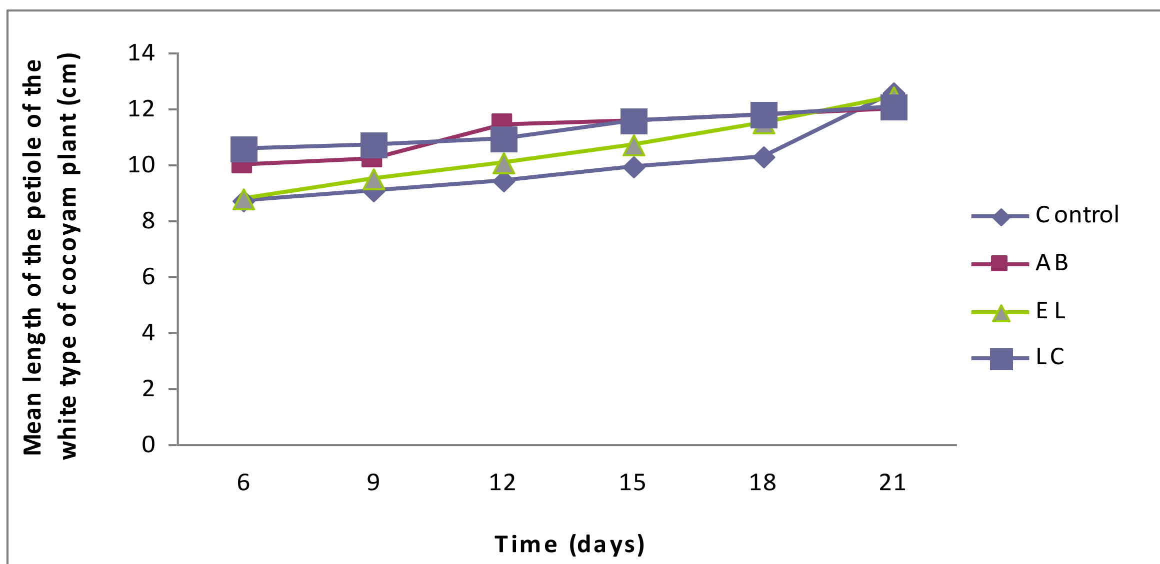


**Fig. 10.** Effect of the three isolates of *S. rolfsii* on the length of the petiole of the white type of cocoyam plant.

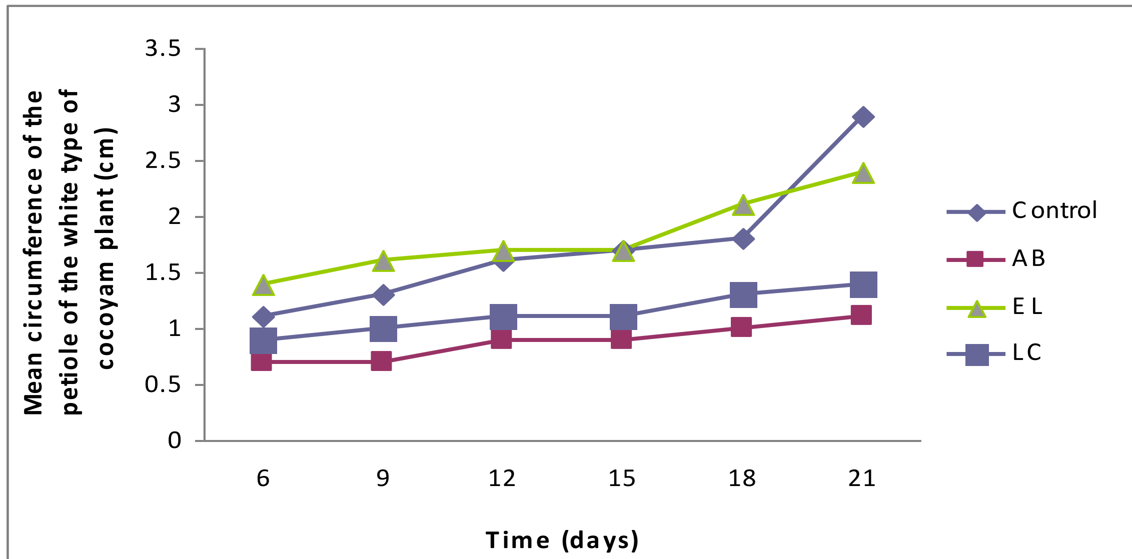


**Fig. 11**. Effect of the three isolates of *S. rolfsii* on the circumference of the petiole of the white type of cocoyam plant.

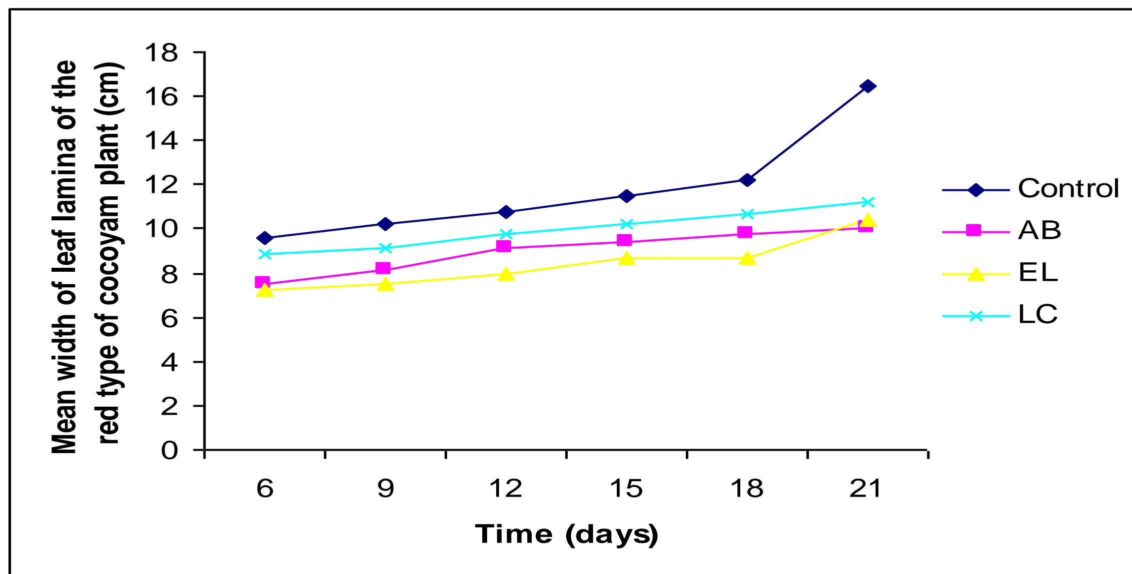


**Fig. 12:** Effect of the three isolates of *S. rolfsii* on the length of the leaf lamina of the red type of cocoyam plant.

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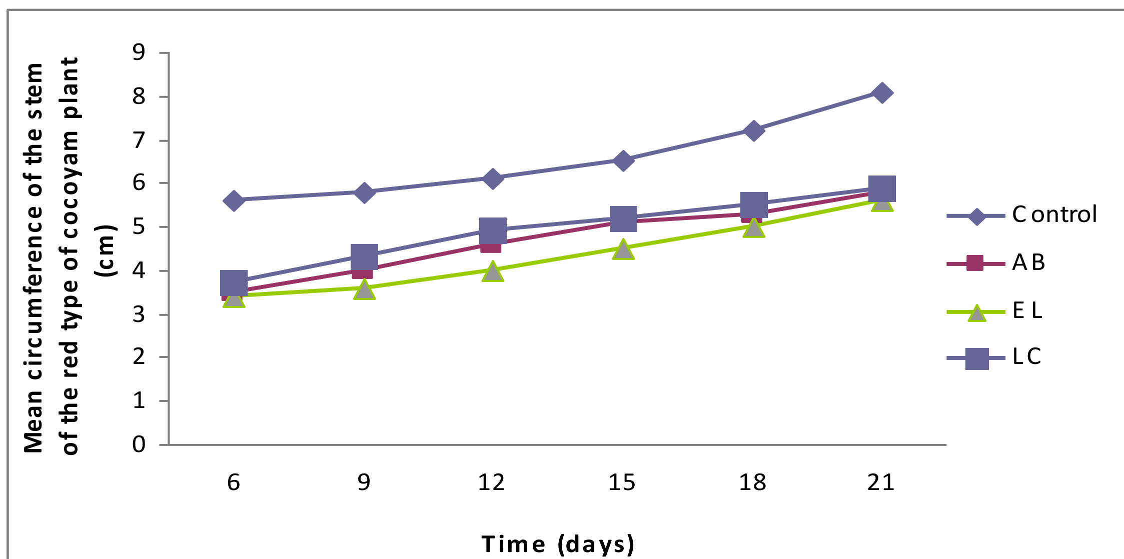


**Fig. 13**. Effect of the three isolates of *S. rolfsii* on the width of leaf lamina of the red type of cocoyam plant.

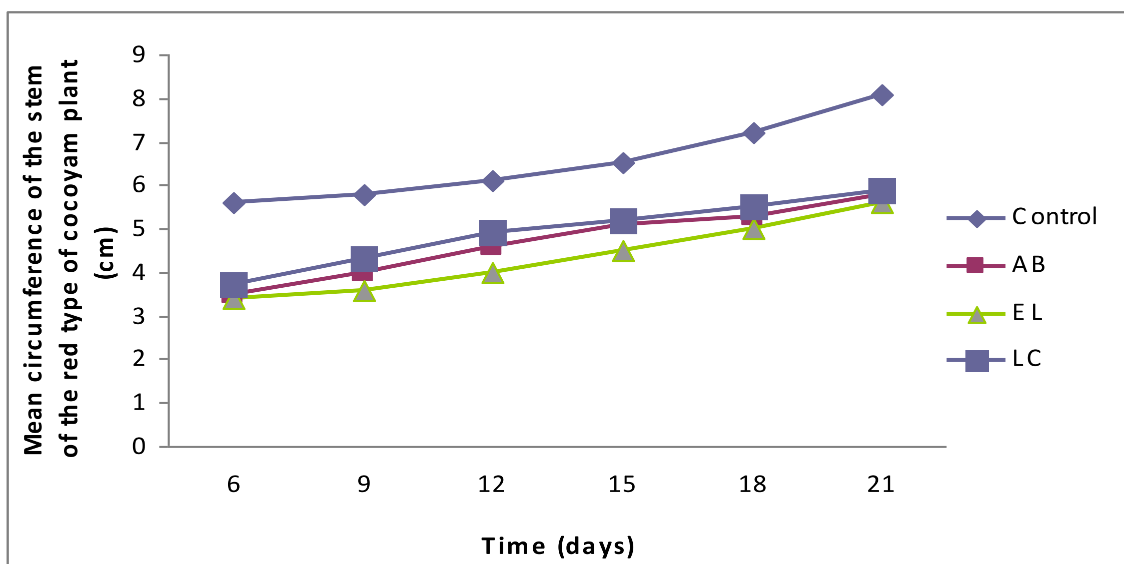


**Fig. 14**. Effect of the three isolates of *S rolfsii* on the circumference of the stem of the red type of cocoyam plant.

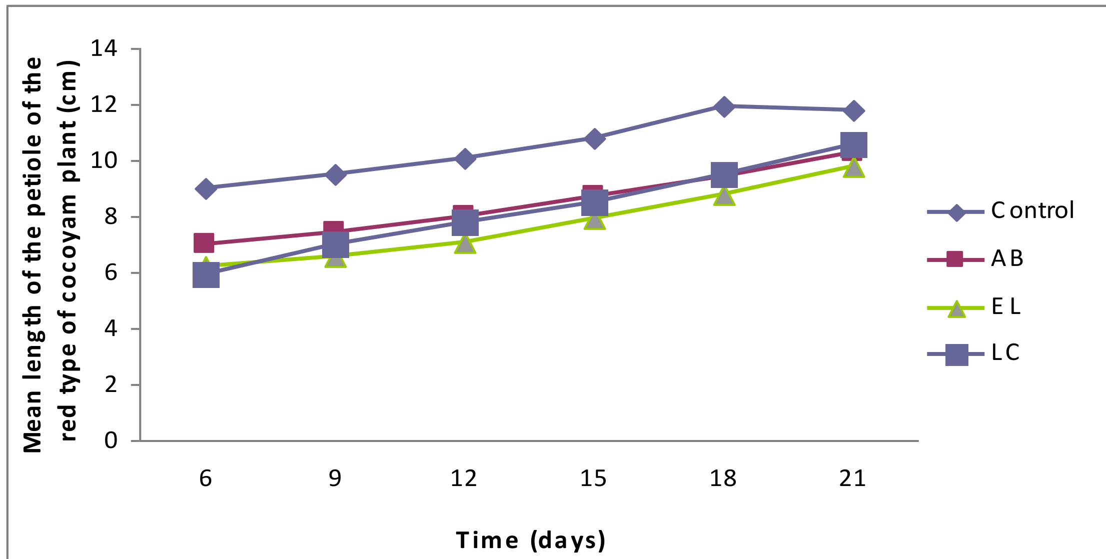


**Fig. 15**. Effect of the three isolates of *S. rolfsii* on the length of the petiole of the red type of cocoyam.

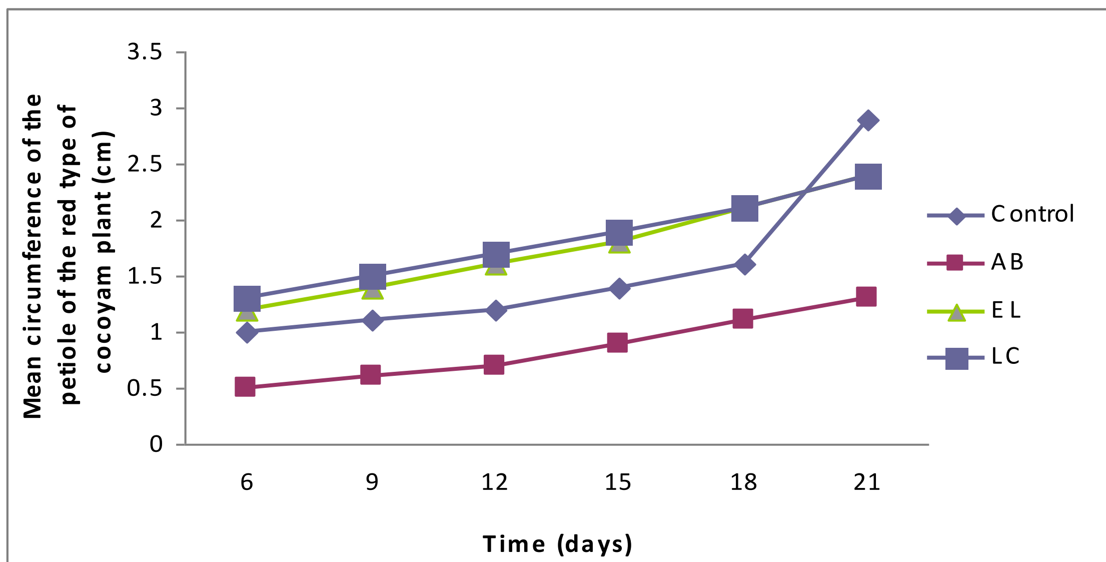


**Fig. 16:**. Effect of the three isolates of *S. rolfsii* on the circumference of the petiole of the red type of cocoyam plant.

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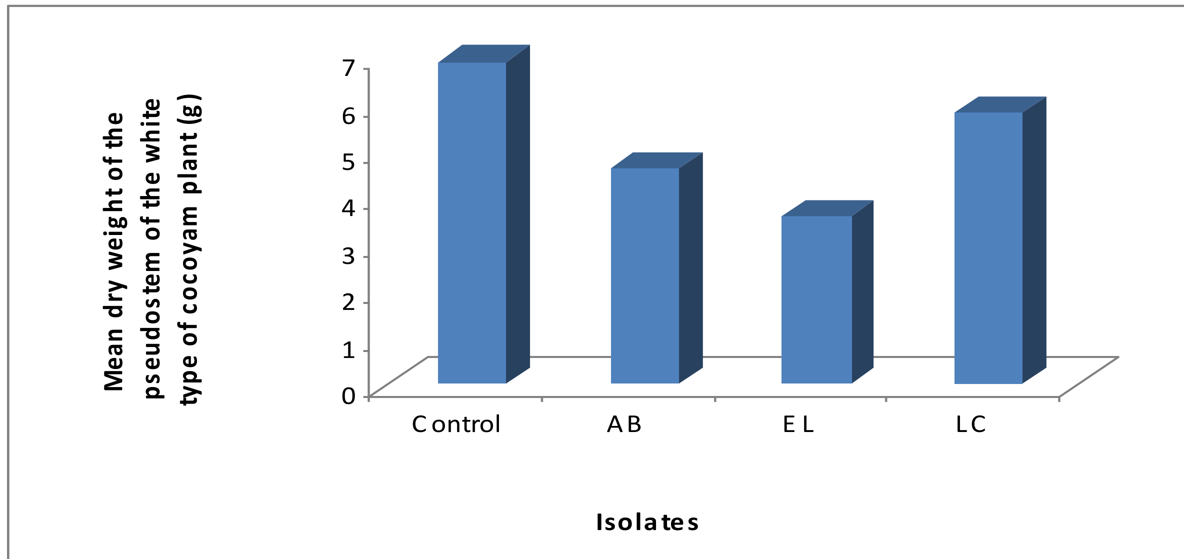


**Fig. 17**. Effect of the three isolates of *S. rolfsii* on dry weight of the pseudostem of the white type of cocoyam plant.

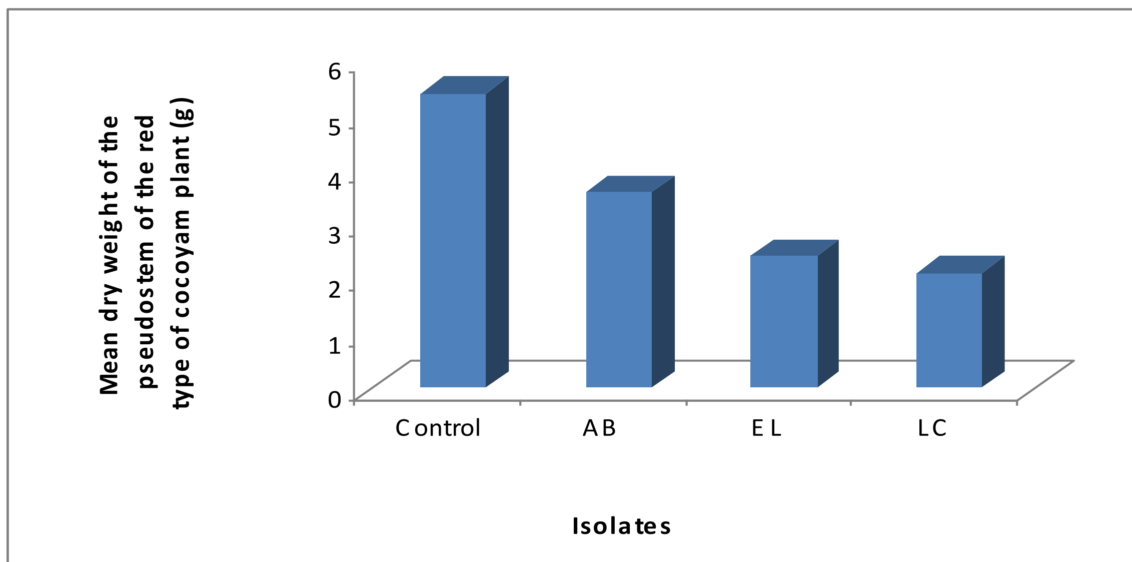


**Fig. 18**. Effect of the three isolates of *S. rolfsii* on the dry weight of the pseudostem of the red type of cocoyam plant

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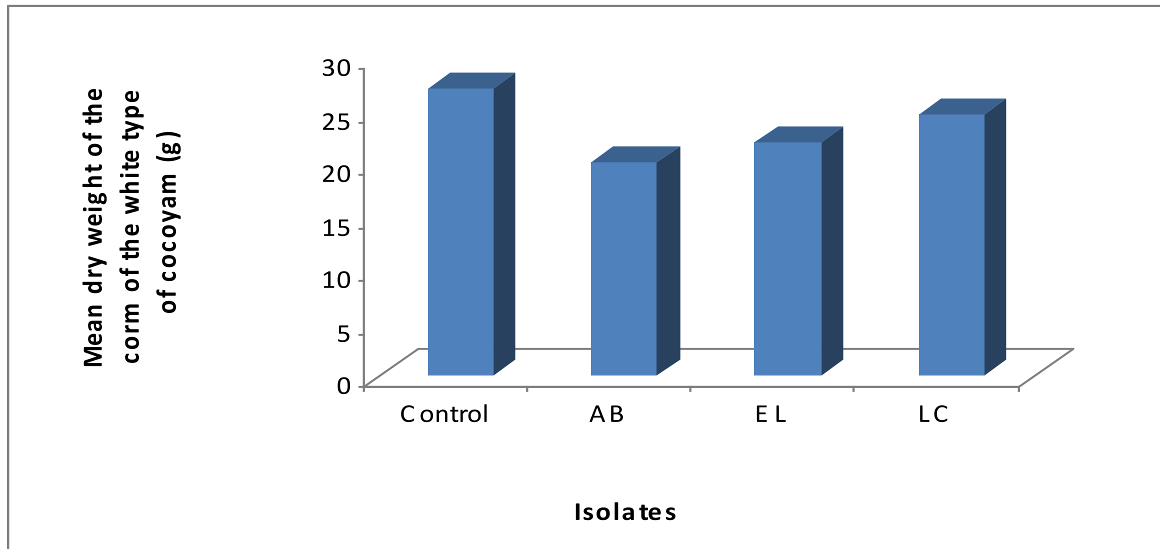


**Fig. 19**. Effect of the three isolates of *S. rolfsii* on the dry weight of the corm of the white type of cocoyam.

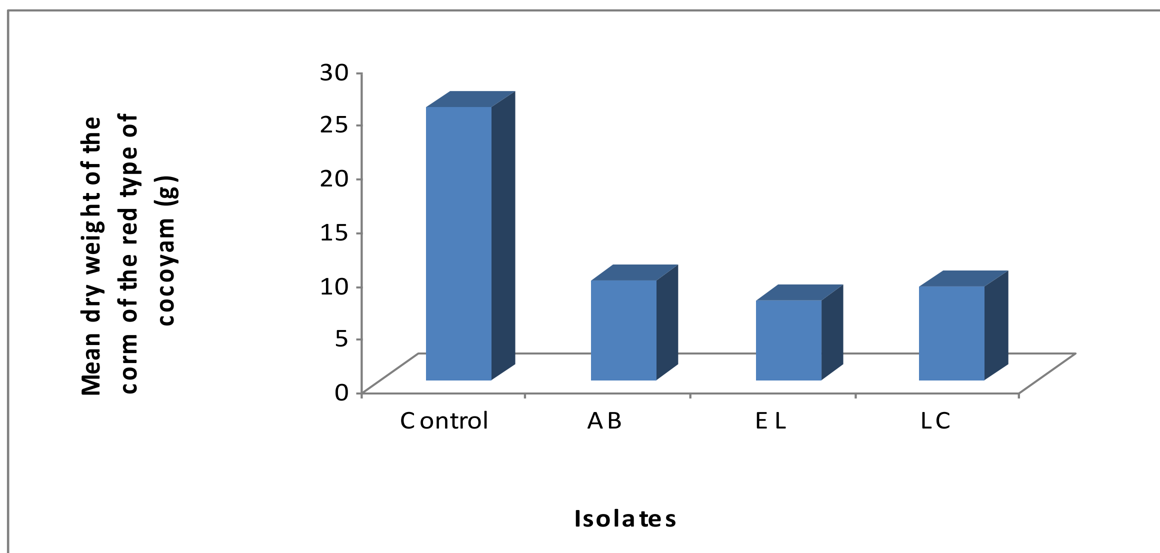


**Fig. 20**. Effect of the three isolates of *S. rolfsii* on the dry weight of the corm of the red type of cocoyam.

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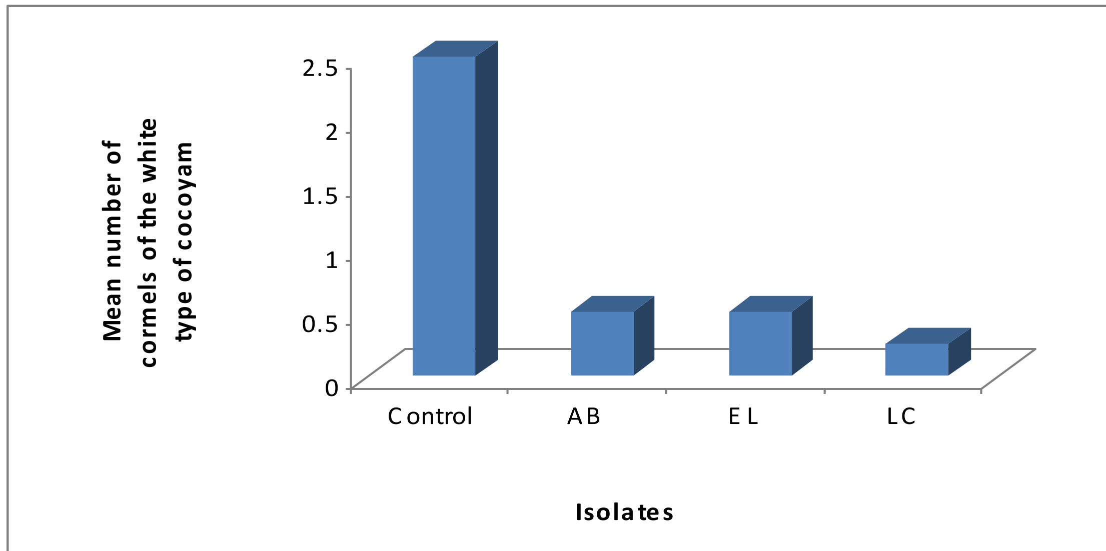


**Fig. 21**. Effect of the three isolates of *S. rolfsii* on the production of cormels of the white type of cocoyam.

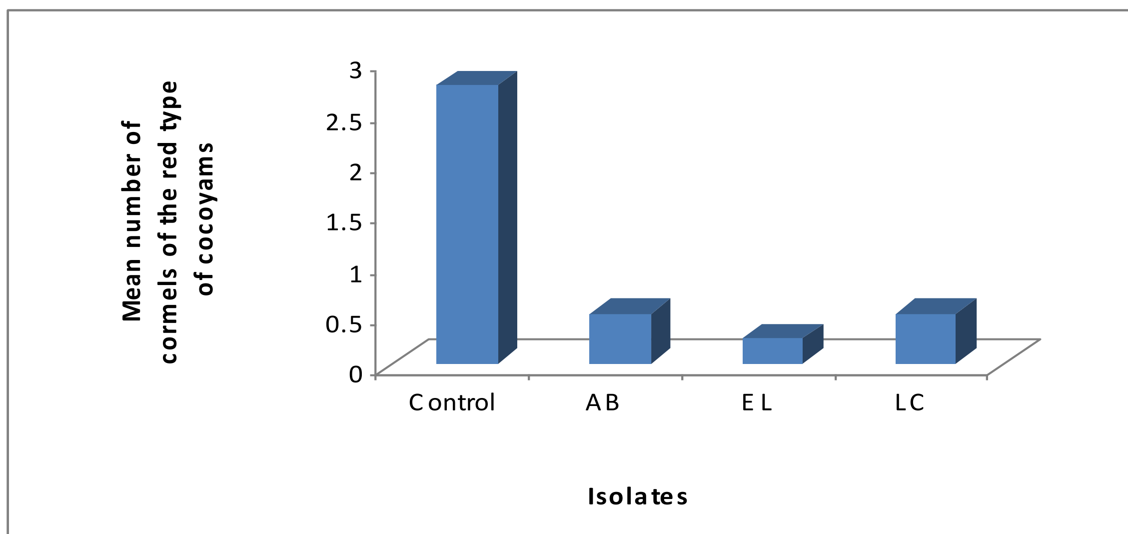


**Fig. 22**. Effect of the three isolates of *S. rolfsii* on the production of cormels of the red type of cocoyam.

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**TABLE 2:** Mean values for phenotypic variables of white cocoyam cultivated in *S. rolfsii* sclerotium infested soil at 30°C for 21 days.

| Isolate | Mean length of leaf lamina (mm) | Mean width of leaf lamina (mm) | Mean circumference of the pseudostem (mm) | Mean length of the petiole (mm) | Mean circumference of the petiole (cm) |
|---|---|---|---|---|---|
| AB | 14.0 ±1.1 | 12.9±1.2 | 6.8±0.3 | 12.0±1.1 | 1.1±0.1 |
| EL | 15.8±0.9 | 14.0±1.5 | 8.5±0.1 | 12.4±1.2 | 2.4±0.2 |
| LC | 14.3±1.2 | 12.5±0.9 | 8.1±0.5 | 12.1±1.1 | 1.4±0.2 |
| Control | 22.5±1.4 | 18.1±1.3 | 7.1±0.8 | 12.6±1.1 | 2.9±0.4 |

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**TABLE 3:** Mean values for phenotypic variables of red cocoyam cultivated in *S rolfsii* sclerotium infested soil at 30°C for 21 days.

| Isolate | Mean length of leaf lamina (mm) | Mean width of leaf lamina (mm) | Mean circumference of the pseudostem (mm) | Mean length of the petiole (mm) | Mean circumference of the petiole (cm) |
|---|---|---|---|---|---|
| AB | 12.7±0.9 | 10.0±0.6 | 5.8±0.4 | 10.3±0.2 | 1.3±0.1 |
| EL | 11.8±0.9 | 10.4±0.7 | 5.6±0.5 | 9.8±0.6 | 2.4±0.2 |
| LC | 13.4±1.0 | 11.2±0.8 | 5.9±0.3 | 10.6±0.2 | 2.4±0.2 |
| Control | 20.8±1.4 | 16.5±1.3 | 8.1±0.5 | 11.8±0.3 | 2.9±0.3 |

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**TABLE 4:** Mean values for yield variables of white cocoyam cultivated in *S rolfsii* sclerotium infested soil at 30°C for 21 days.

| Isolate | Mean dry weight of pseudostem (mg) | Mean dry weight of corm (mg) | Mean number of cornels produced |
|---|---|---|---|
| AB | 4.6± 0.6 | 20.1±0.4 | 1±0.1 |
| EL | 3.6±0.2 | 21.9±0.8 | 1±0.5 |
| LC | 5.8±0.2 | 24.6±0.7 | 0±0.0 |
| Control | 6.9±0.2 | 27.1±1.2 | 3±0.7 |

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**TABLE 5:** Mean values for yield variables of red cocoyam cultivated in *S rolfsii* sclerotium infested soil at 30℃ for 21 days

| Isolate | Mean dry weight of shoot (mg) | Mean dry weight of corm (mg) | Mean number of cormels produced |
|---|---|---|---|
| AB | 3.6±0.2 | 9.4±0.7 | 1±0.2 |
| EL | 2.4±0.1 | 7.5±0.4 | 0±0.0 |
| LC | 2.1±0.3 | 8.9±1.4 | 1±0.2 |
| Control | 5.4±0.6 | 25.7±2.1 | 3±0.2 |

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## 5 : EFFECT OF HEAT- STERILIZED ETHANOL LEAF EXTRACTS OF PLANTAIN ON THE RADIAL GROWTH OF MYCELIA AND PRODUCTION OF SCLEROTIA BY THREE ISOLATES OF *SCLEROTIUM ROLFSII.*

The radial growth of the three different isolates of *S. rolfsii* AB, EL and LC were depressed initially by the various dilution levels with the greatest depression being produced by the undiluted solution followed by the 1:1v/v, 1:2 v/v and 1:5 v/v dilutions in that order (Fig. 23). The greater the concentration, the greater the depressive effect and that at the maximum duration of the study, the radial growth for the 1:5 v/v dilution level for the LC isolate equaled that of the control. For all the dilution levels, the AB isolate as compared to the other isolates grew slowlest followed by the EL and the LC isolates in that order (Fig. 23). Sclerotium production was more inhibited in the AB isolate than in the EL and the LC isolates (Fig. 24).

The undiluted plantain extract was able to reduce radial growth of mycelia of the AB, EL and LC isolates by 67.1%, 57.8% and 30.47% respectively after 10 days of incubation and sclerotium production by 28, 29 and 35 in cultures of isolates AB, EL and LC respectively after 14 days of incubation (Table 6).

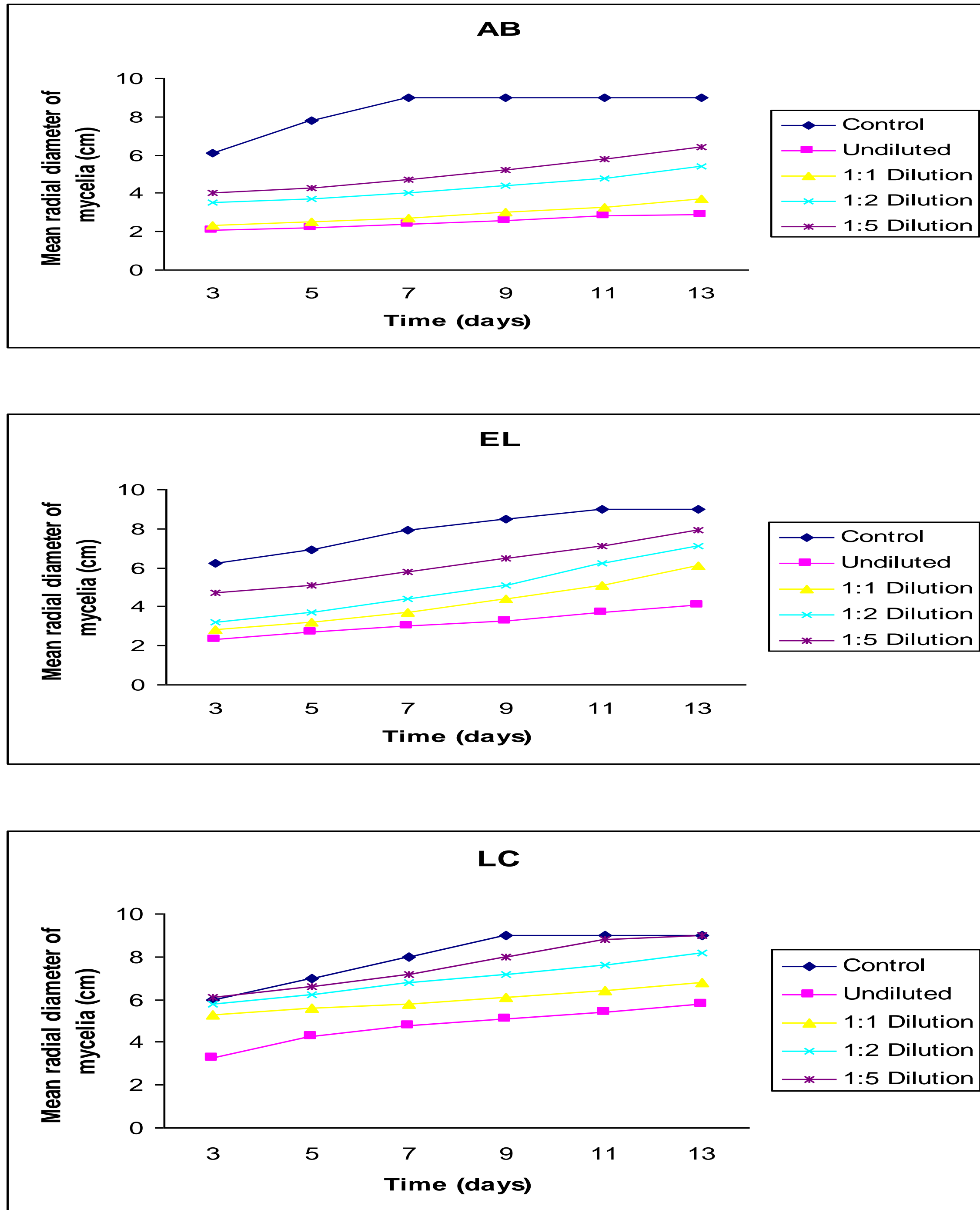


**Fig. 23.** Effect of ethanol leaf extract of plantain in PDA on radial growth of the three isolates of *S. rolfsii* at 28ºC.

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**TABLE 6 :** Fungitoxic effect of ethanol leaf extracts of plantain in PDA on *S rolfsii* growth and sclerotium production after 10 and 14 days of incubation respectively at 28ºC.

| Concentration (V/V) | Percentage (%) growth Inhibition of isolate | | | Mean number of sclerotia per petri plate produced | | |
|---|---|---|---|---|---|---|
| | AB | EL | LC | AB | EL | LC |
| Undiluted | 67.10 | 57.80 | 30.47 | 28±2.1 | 29±2.2 | 35±2.1 |
| 1:1 | 61.62 | 44.15 | 12.79 | 74±2.2 | 78±1.9 | 80±2.1 |
| 1:2 | 43.43 | 34.43 | 1.26 | 93±2.2 | 98±2.0 | 101±1.9 |
| 1;5 | 33.33 | 18.10 | 1.07 | 134±1.8 | 141±1.7 | 142±1.7 |
| Control | 0 | 0 | 0 | 389±1.9 | 473±1.7 | 477±1.4 |

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## 6 : EFFECT OF HEAT- STERILIZED ETHANOL LEAF EXTRACTS OF CASSAVA ON THE RADIAL GROWTH OF MYCELIA AND PRODUCTION OF SCLEROTIA BY THREE ISOLATES OF *SCLEROTIUM ROLFSII.*

In Experiment 5, the radial growth of the three isolates of *S. rolfsii* was depressed in response to the phytotoxins present in the leaf extracts of cassava. The same trend of depressed radial growth was again observed in the three isolates AB, EL and LC in response to the phytotoxins present in the leaf extract of cassava. A critical analysis of the results reveals that the radial growth of mycelia for the various dilution levels of undiluted, 1:1 v/v, 1:2 v/v and 1:5 v/v was more depressed in the leaf extracts of cassava than of plantain (Fig 25). Sclerotiium production was more reduced in the EL isolate than in the AB and LC isolates (Fig. 26).

The undiluted extract of cassava was depressed by 67.6%, 45.3% and 55.6% in AB, EL and LC respectively after 10 days of incubation and suppressed sclerotia production of 36 , 43 and 52 sclerotia by the isolates AB, EL and LC respectively after 14 days of incubation (Table 7).

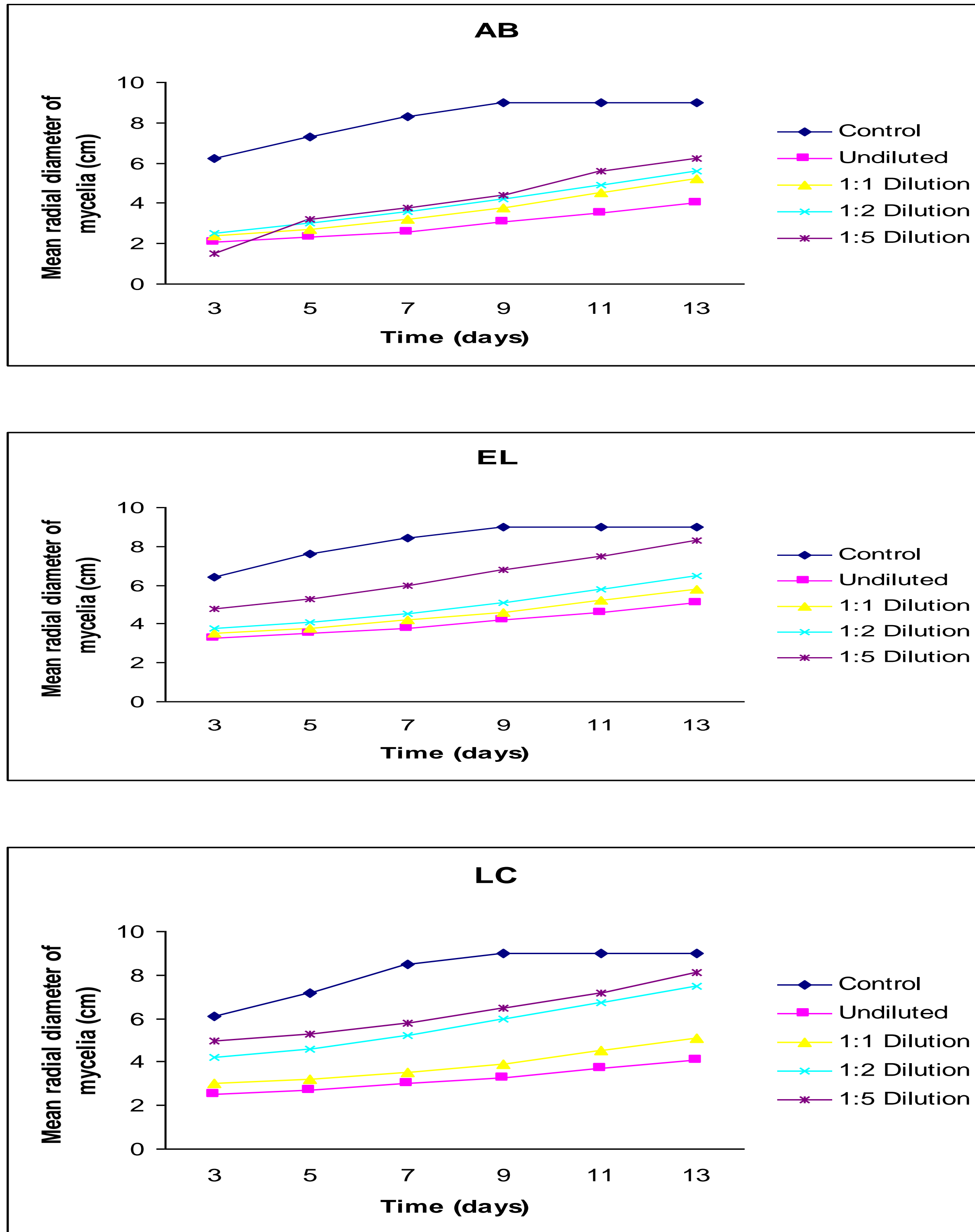


**Fig. 24**. Effect of ethanol leaf extract of cassava on radial growth of the three isolates of *S. rolfsii* on PDA at 28°C.

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**TABLE 7 :** Fungitoxic effect of ethanol leaf extracts of cassava in PDA on *S rolfsii* growth and sclerotium production after 10 and 14 days of incubation respectively at 28ºC.

| Concentration (V/V) | Percentage (%) growth Inhibition of isolate | | | Mean number of sclerotia per petri plate produced | | |
|---|---|---|---|---|---|---|
| | AB | EL | LC | AB | EL | LC |
| Undiluted | 67.61 | 45.36 | 55.60 | 36±0.9 | 45±0.8 | 52±0.7 |
| 1:1 | 51.42 | 40.01 | 47.21 | 94±1.2 | 97±1.1 | 91±1.1 |
| 1:2 | 45.92 | 33.31 | 44.40 | 123±1.2 | 134±1.1 | 134±1.2 |
| 1;5 | 36.52 | 14.71 | 36.10 | 178±2.1 | 181±1.1.9 | 174±1.8 |
| Control | 0 | 0 | 0 | 505±2.1 | 493±1.9 | 519±1.8 |

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## 7: EFFECT OF HEAT- STERILIZED ETHANOL LEAF EXTRACTS OF COCOYAM ON THE RADIAL GROWTH OF MYCELIA AND PRODUCTION OF SCLEROTIA BY THREE ISOLATES OF *SCLEROTIUM ROLFSII.*

As with the ethanol leaf extracts of plantain and cassava, there was a marked variation in the responses of the various isolates to the active ingredients of fungitoxic nature present in the cocoyam leaf extract.. As observed in the extracts of plantain and cassava, the inhibitory effect of the active ingredients or phytotoxins was gradually removed as dilution increased. For the maximum duration of the study, the radial growth of the AB isolate in the heat-sterilized ethanol leaf extract of cocoyam equalled that of cassava for the 1:5 v/v dilution level (Fig 27) in as much as 1:2 v/v dilution level for both LC isolate and the AB isolate (Fig. 28).

The percentage growth inhibition of radial growth of the three isolates AB, EL and LC by the undiluted leaf extract of cocoyam was 66.30%, 64.37% and 54.36% respectively after 10 days of incubation and the mean number of sclerotia produced was 21, 23 and 26 respectively after 14 days of incubation (Table 8)

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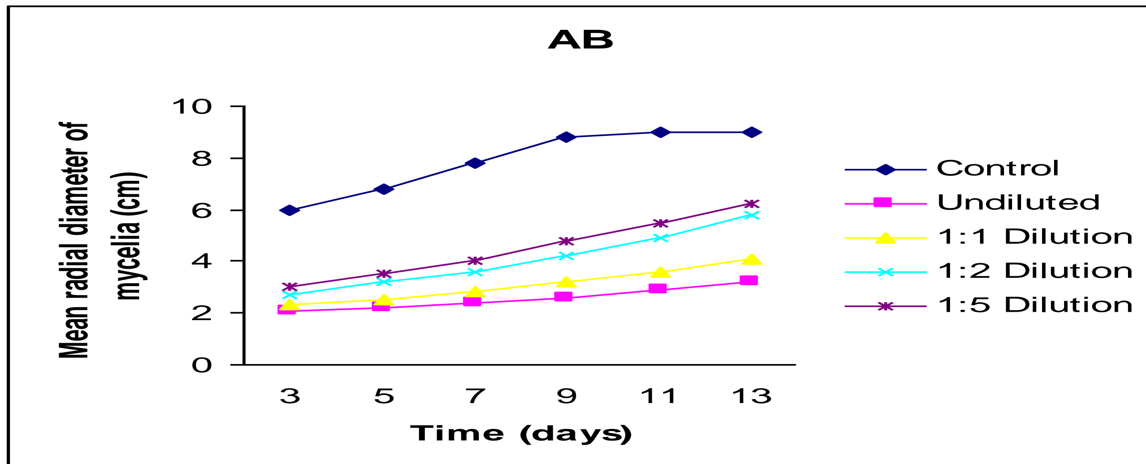


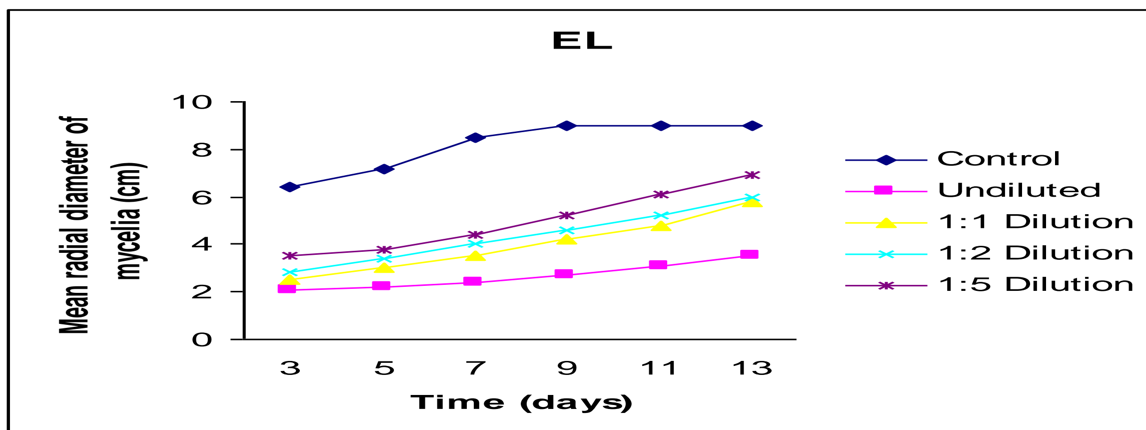


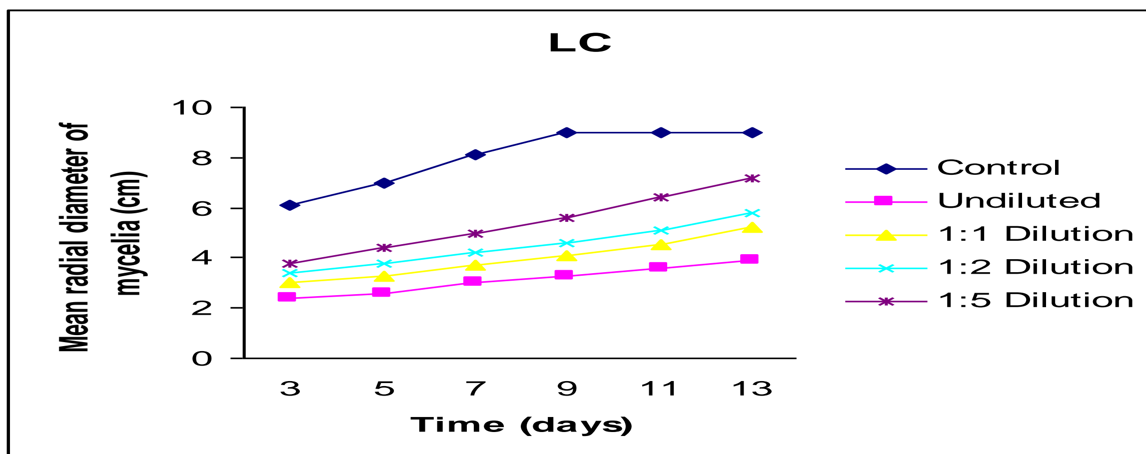


**Fig. 25**. Effect of ethanol leaf extract of cocoyam in PDA on radial growth of the three isolates of *S. rolfsii* on PDA at 28ºC..

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**TABLE 8** Fungitoxic effect of ethanol leaf extracts of cocoyam in PDA on *S rolfsii* growth and sclerotium production after 10 and 14 days of incubation respectively at 28ºC.

| Concentration (V/V) | Percentage (%) growth Inhibition of isolate | | | Mean number of sclerotia per petri plate produced | | |
|---|---|---|---|---|---|---|
| | AB | EL | LC | AB | EL | LC |
| Undiluted | 66.30 | 64.37 | 54.36 | 21±0.4 | 26± 0.3 | 23±0.2 |
| 1:1 | 59.47 | 47.50 | 42.29 | 37±0.4 | 38±0.6 | 39±1.0 |
| 1:2 | 46.44 | 42.50 | 34.74 | 106±1.2 | 118±1.4 | 113±1.4 |
| 1;5 | 40.66 | 40.66 | 33.58 | 124±0.9 | 164±2.2 | 160±1.7 |
| Control | 0 | 0 | 0 | 500±2.1 | 515±2.1 | 504±1.9 |

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## 8: EFFECT OF HEAT- STERILIZED AQUEOUS LEAF EXTRACTS OF PLANTAIN ON THE RADIAL GROWTH OF MYCELIA AND PRODUCTION OF SCLEROTIA BY THREE ISOLATES OF *SCLEROTIUM ROLFSII.*

Radial growth of mycelia of the three isolates commenced after 24 hours and there was no "lag phase" as in the experiments involving the ethanol extracts in which growth commenced on the third day (Fig. 29). Another observation was that the inhibitory effect was greatly reduced as compared to the heat-sterilized ethanol leaf extracts. Just as observed in the heat-sterilized leaf extract of ethanol, radial growth of mycelia of AB, EL and LC isolates increased as a result of a reduction in the inhibitory effect with increasing dilution. For the 1:5 v/v dilution level, all the three isolates AB, EL and LC equaled that of the phytotoxin-free control for the maximum duration of the study (Fig 29) in as much as 1:2 v/v level for the AB and LC isolates. There was less repression in sclerotia development for the aqueous extract as compared to the ethanol extract (Fig. 32).

The percentage growth inhibition of the radial growth of mycelia of the isolates by the undiluted aqueous extract of plantain was 66.30%, 64.17% and 54.36% for isolates AB, EL and LC respectively after 10 days of incubation and the mean number of sclerotia produced was 50, 65 and 85 respectively after 14 days of incubation.(Table 9).

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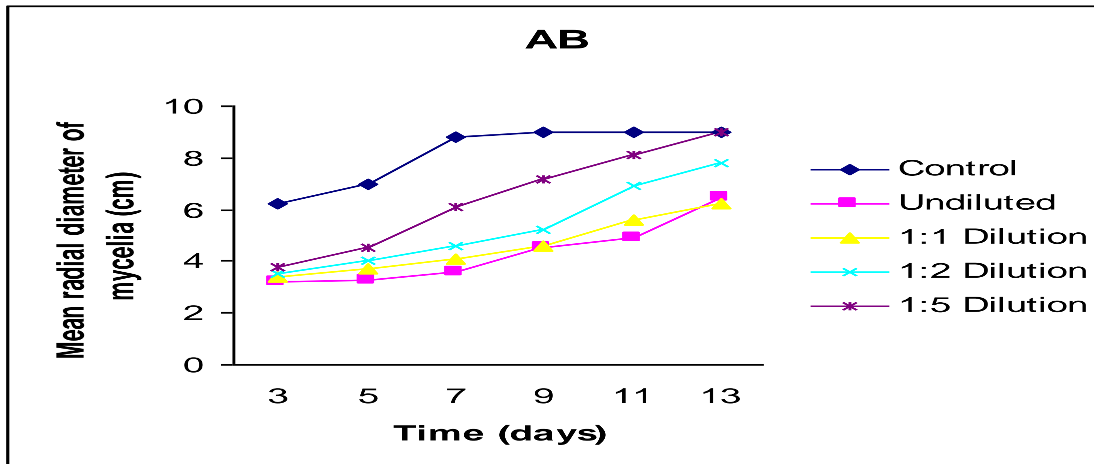


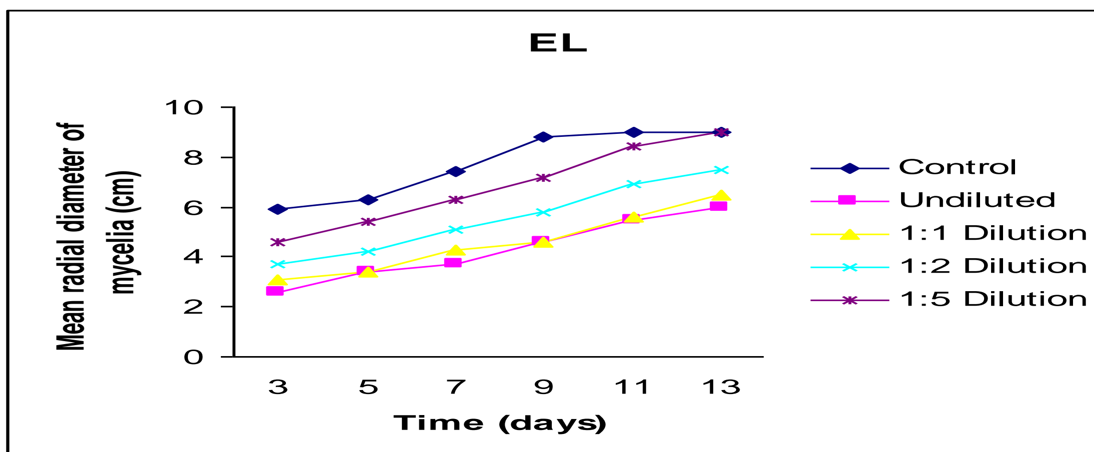


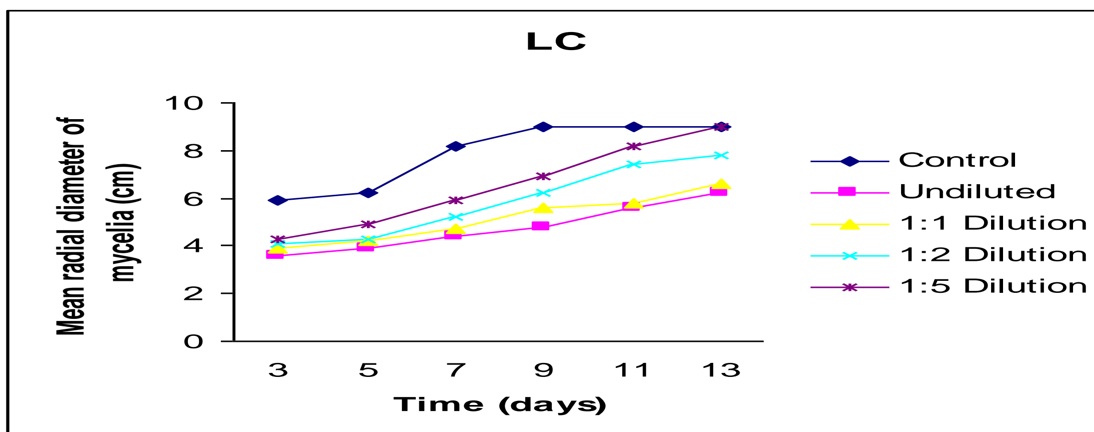


**Fig. 26**. Effect of aqueous leaf extract of plantain in PDA on radial growth of the three isolates of *S. rolfsii* on PDA at 28ºC.

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**TABLE 9 :** Fungitoxic effect of aqueous leaf extract of plantain in PDA on *S rolfsii* growth and sclerotium production after 10 and 14 days of incubation respectively at 28ºC.

| Concentration (V/V) | Percentage (%) growth Inhibition of isolate | | | Mean number of sclerotia per petri plate produced | | |
|---|---|---|---|---|---|---|
| | AB | EL | LC | AB | EL | LC |
| Undiluted | 43.03 | 44.11 | 30.96 | 50±0.9 | 85±1.1 | 65±0.8 |
| 1:1 | 39.47 | 40.66 | 25.29 | 101±1.1 | 126±1.3 | 117±1.4 |
| 1:2 | 29.74 | 26.75 | 14.97 | 144±1.8 | 155±2.1 | 144±1.9 |
| 1;5 | 16.71 | 9.70 | 7.40 | 237±2.2 | 248±2.3 | 237±2.1 |
| Control | 0 | 0 | 0 | 528±2.4 | 537±2.5 | 513±2.8 |

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## 9 : EFFECT OF HEAT- STERILIZED AQUEOUS LEAF EXTRACT OF CASSAVA ON THE RADIAL GROWTH OF MYCELIA AND PRODUCTION OF SCLEROTIA BY THREE ISOLATES OF *SCLEROTIUM ROLFSII.*

The repressive effect of the fungitoxic phytotoxins present in the heat-sterilized aqueous leaf extract of cassava basically followed the same trend as that of the plantain extract. The radial mycelial growth of the three isolates AB, EL and LC equaled that of the phytotoxin-free control for the maximum duration of the study for the 1:5 v/v dilution level (Fig. 31). Inhibition of sclerotia production was reduced as compared to the ethanol extract (Fig.32).

The percentage growth inhibition of the radial growth of mycelia of the isolates by the undiluted aqueous extract of cassava leaves was 42.89%,43.71% and 30.71% for isolates AB, EL and LC respectively after 10 days of incubation and the mean number of sclerotia produced was 44, 71 and 82 respectively after 14 days of incubation (Table 10).

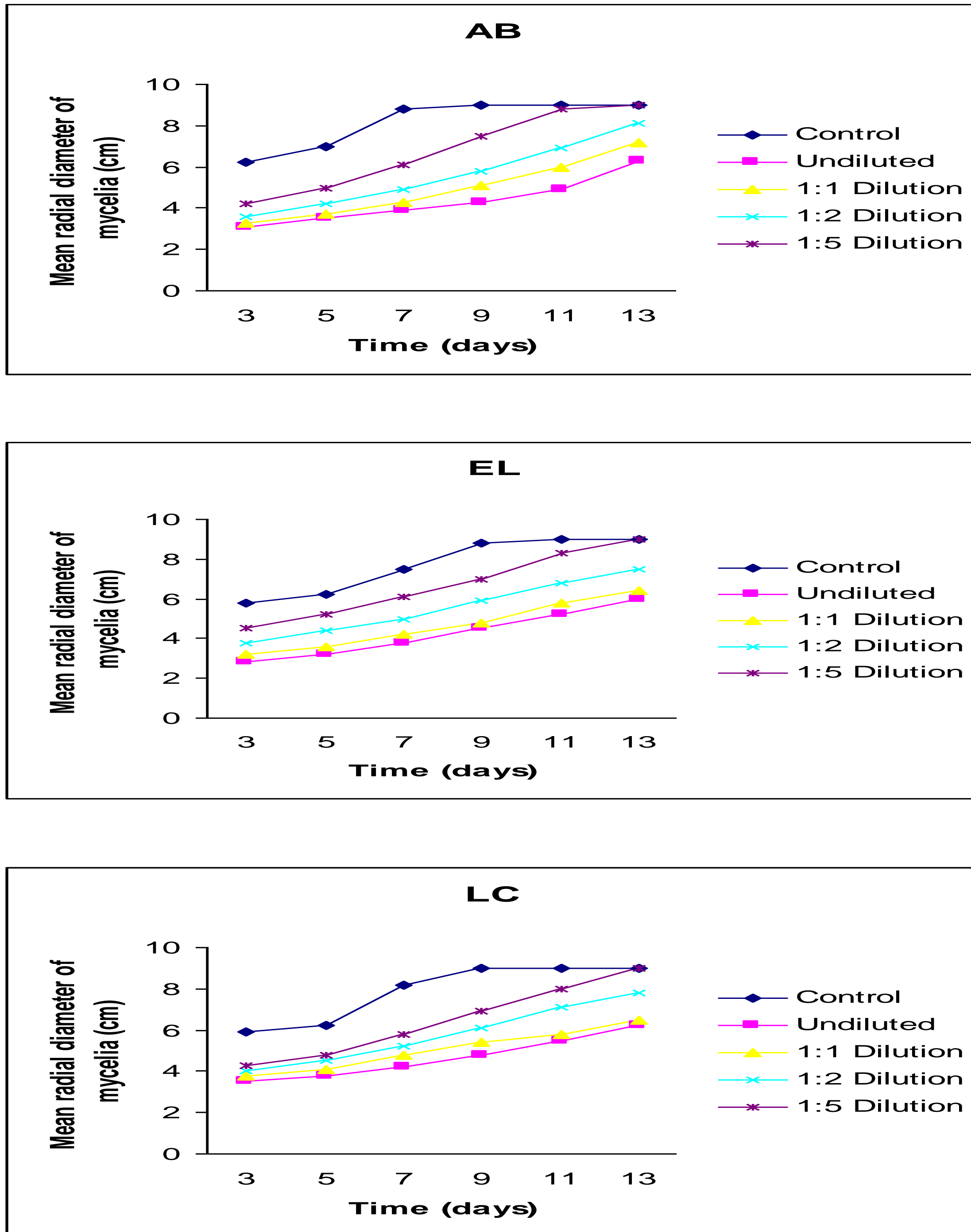


**Fig. 27**. Effect of aqueous leaf extract of cassava in PDA on radial growth of the three isolates of *S. rolfsii* on PDA at 28ºC..

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**TABLE 10** : Fungitoxic effect of aqueous leaf extract of cassava in PDA on *S rolfsii* growth and sclerotium production after 10 and 14 days of incubation respectively at 28ºC.

| Concentration (V/V) | Percentage (%) growth Inhibition of isolate | | | Mean number of sclerotia per petri plate produced | | |
|---|---|---|---|---|---|---|
| | AB | EL | LC | AB | EL | LC |
| Undiluted | 42.89 | 43.71 | 30.71 | 44±0.4 | 82±0.6 | 71±0.5 |
| 1:1 | 37.28 | 38.19 | 26.36 | 102±1.1 | 124±1.4 | 113±1.2 |
| 1:2 | 26.53 | 26.27 | 15.94 | 140±2.1 | 153±2.2 | 136±1.6 |
| 1;5 | 10.96 | 11.48 | 6.00 | 229±2.3 | 235±2.2 | 229±1.9 |
| Control | 0 | 0 | 0 | 546±2.7 | 538±2.4 | 541±2.5 |

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## 10: EFFECT OF HEAT- STERILIZED AQUEOUS LEAF EXTRACT OF COCOYAM ON THE RADIAL GROWTH OF MYCELIA AND PRODUCTION OF SCLEROTIA BY THREE ISOLATES OF *SCLEROTIUM ROLFSII.*

There was a little variation in the responses of the isolates AB, EL and LC of *Sclerotium rolfsii* to the phytotoxins present in the heat-sterilized leaf extract of cocoyam. The same trend of a reduction in the repressive effect with increasing dilution was observed (Fig. 33). There was less inhibition of sclerotia production when compared to the ethanol extract (Fig. 34).

The percentage growth inhibition of the radial growth of mycelia of the isolates by the undiluted aqueous extract of cocoyam leaves was 49.56%, 44.59% and 43.13% for isolates AB , EL and LC respectively after 10 days of incubation and the number of sclerotia produced was 55, 71 and 65 respectively after 14 days of incubation (Table 11).

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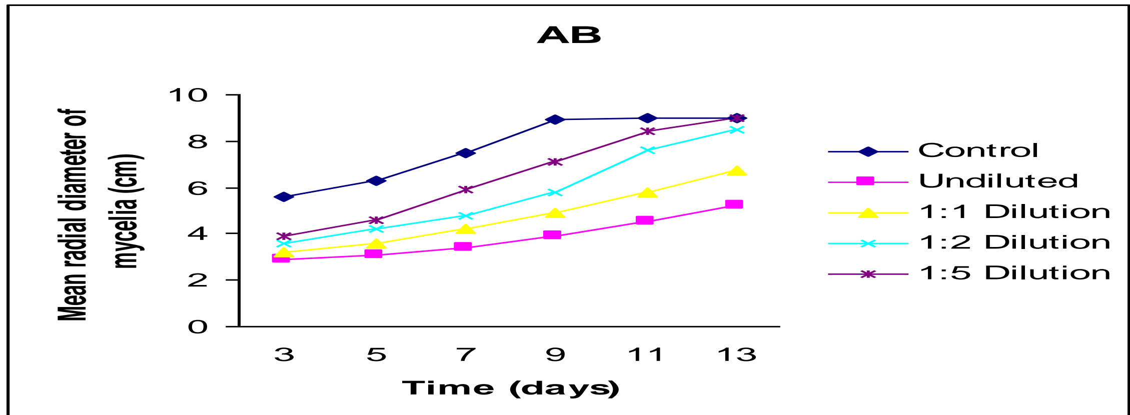


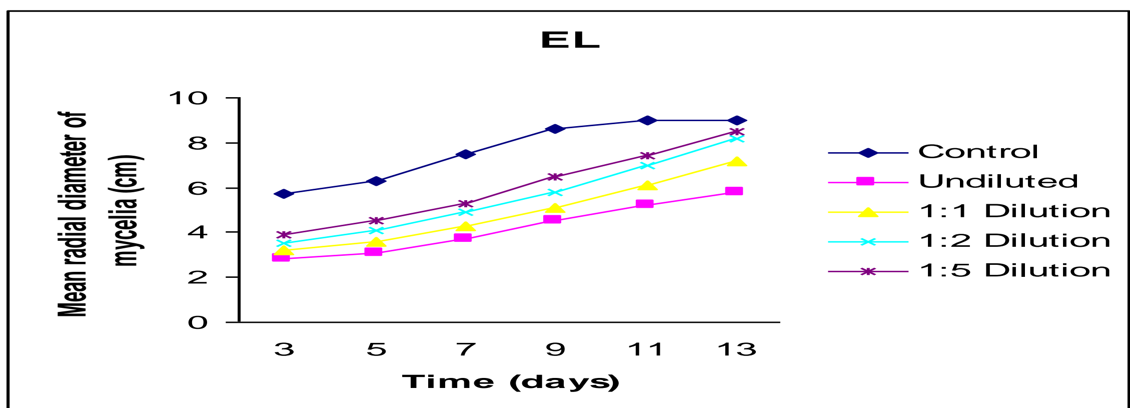


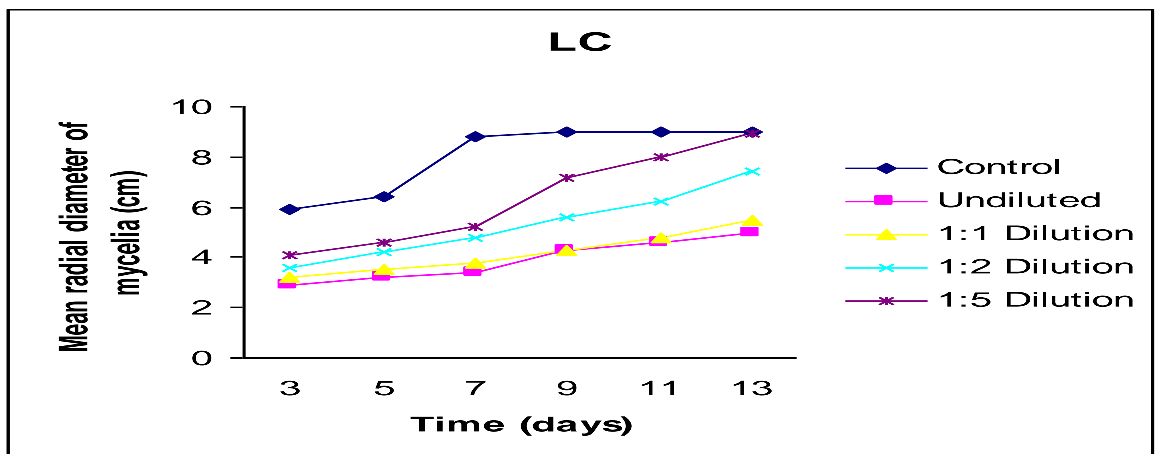


**Fig. 28**. Effect of aqueous leaf extract of cocoyam in PDA on radial growth of the three isolates of *S. rolfsii* on PDA at 28ºC.

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**TABLE 11** : Fungitoxic effect of aqueous leaf extract of cocoyam in PDA on *S rolfsii* growth and sclerotium production after 10 and 14 days of incubation respectively at 28ºC.

| Concentration (V/V) | Percentage (%) growth Inhibition of isolate. | | | Mean number of sclerotia per petri plate produced. | | |
|---|---|---|---|---|---|---|
| | AB | EL | LC | AB | EL | LC |
| Undiluted | 49.56 | 44.59 | 43.13 | 55±0.4 | 71±0.8 | 65±0.6 |
| 1:1 | 29.82 | 34.80 | 39.19 | 83±0.6 | 111±1.2 | 111±1.3 |
| 1:2 | 24.30 | 26.05 | 22.97 | 131±1.4 | 138±1.6 | 131±1.3 |
| 1;5 | 14.69 | 20.30 | 7.90 | 220±2.1 | 221±2.1 | 215±1.9 |
| Control | 0 | 0 | 0 | 580±2.4 | 575±2.2 | 554±2.3 |

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## 11: STUDIES ON EFFECT OF HEAT-STERILIZED ETHANOL LEAF EXTRACT OF PLANTAIN ON THE VEGETATIVE GROWTH OF MYCELIA OF THE THREE ISOLATES OF *SCLEROTIUM ROLFSII*.

The mycelia of the various isolates of *Sclerotium rolfsii* AB, EL and LC exhibited varied responses to the heat-treated fungitoxic phytotoxins present in the leaf extract of plantain and that the inhibition of the vegetative growth was basically commensurate with the concentration of the heat-sterilized leaf extract. The depression in vegetative growth was highest in the undiluted extract which did not produce any mycelial growth for all the isolates AB, EL and LC. However despite the fact that the inhibitory effect reduced with increasing dilution, none of the isolates approximated that of the control (Broth only) not even for the least concentration of 1:5 v/v for the maximum duration of the study (Fig. 35).

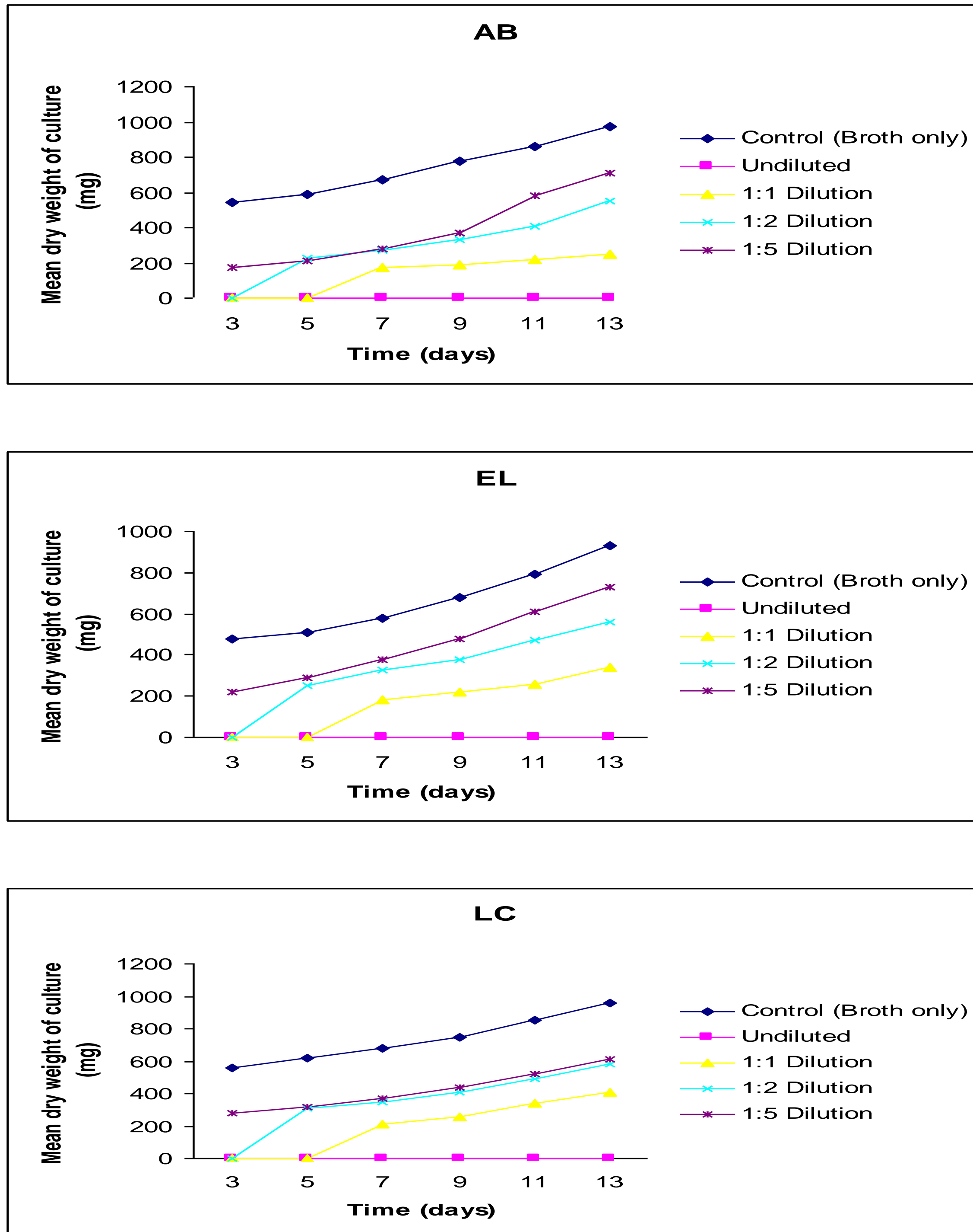


**Fig. 29.** Effect of ethanol leaf extract of plantain in PDB on the vegetative growth of the three isolates of *S. rolfsii* at 28ºC.

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## 12: STUDIES ON EFFECT OF HEAT-STERILIZED ETHANOL LEAF EXTRACT OF CASSAVA ON THE VEGETATIVE GROWTH OF MYCELIA OF THE THREE ISOLATES OF *SCLEROTIUM ROLFSII*.

The depression in growth of the mycelia of the various isolates AB, EL and LC was also manifested in the heat-sterilized leaf extract of cassava and that the vegetative growth of mycelia was linked to the concentration of the heat-sterilized extract. For the heat-sterilized extract of cassava, there was limited growth of mycelia in the undiluted extract by the LC isolate though the EL and AB isolates did not produce any growth in the undiluted extract (Fig. 36). In addition the fungitoxic phytotoxins present in the leaf extract of cassava produced a relatively depressed growth of mycelia than the heat-sterilized extract of plantain.

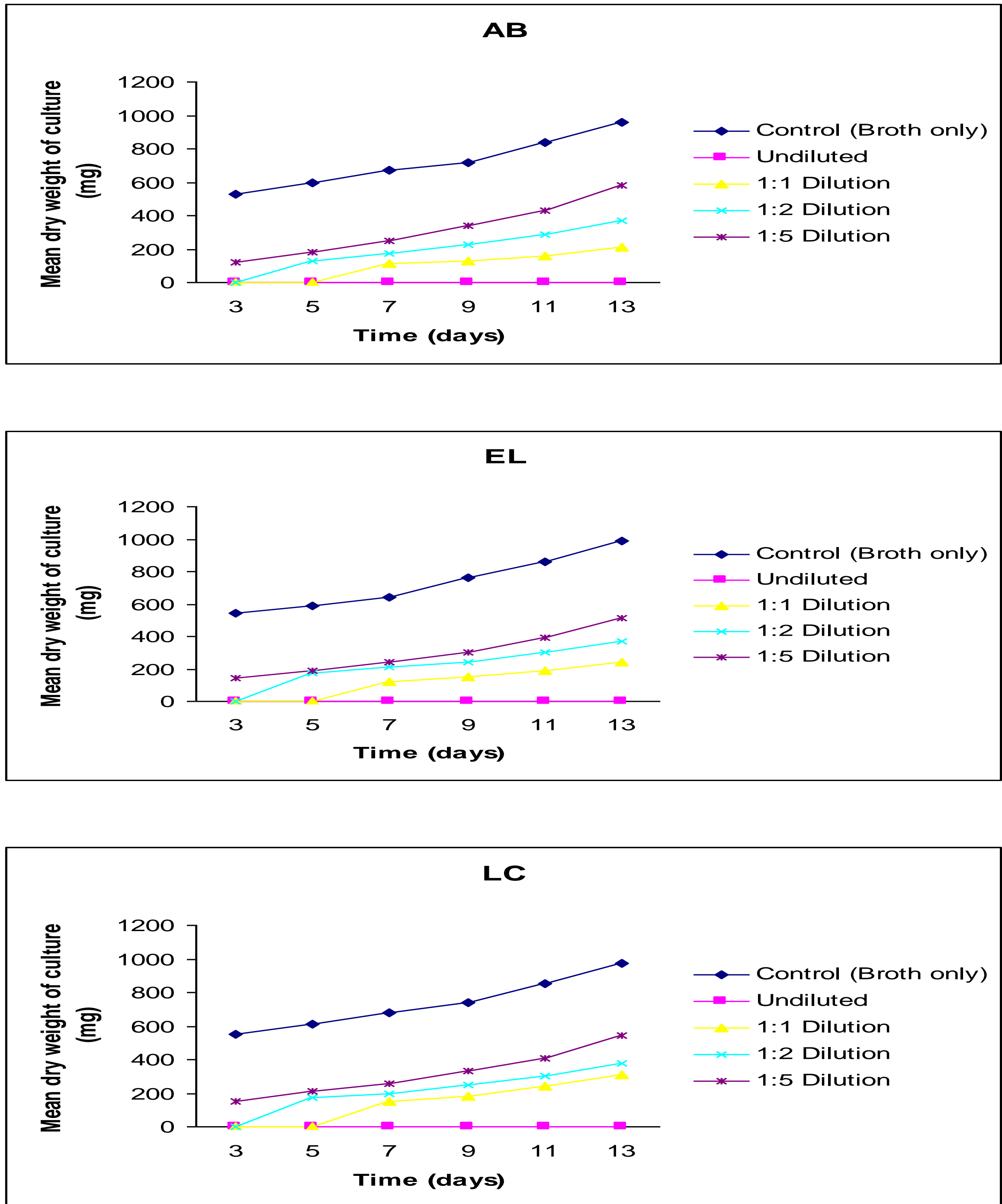


**Fig. 30.** Effect of ethanol leaf extract of cassava in PDB on vegetative growth of the three isolates of *S. rolfsii* at 28ºC.

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## 13: STUDIES ON EFFECT OF HEAT-STERILIZED ETHANOL LEAF EXTRACT OF COCOYAM ON THE VEGETATIVE GROWTH OF MYCELIA OF THE THREE ISOLATES OF *SCLEROTIUM ROLFSII*.

The heat-sterilized ethanol leaf extract of cocoyam was more repressive on the mycelia growth of the three isolates AB, EL and LC comparatively. Expectedly the repressive nature of the undiluted extract was manifested as no growth was recorded for all the isolates. With increasing dilution however, growth was recorded with the least growth occurring in the AB isolate (Fig. 37 and Plate 3).

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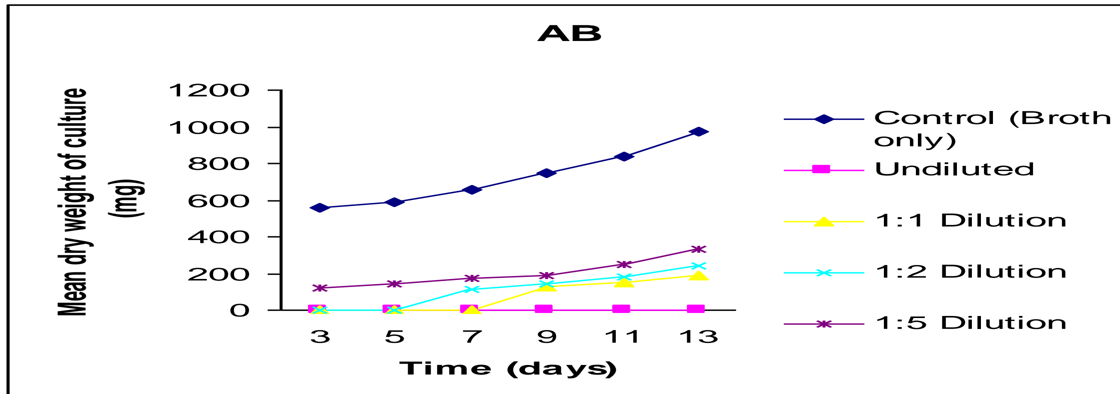


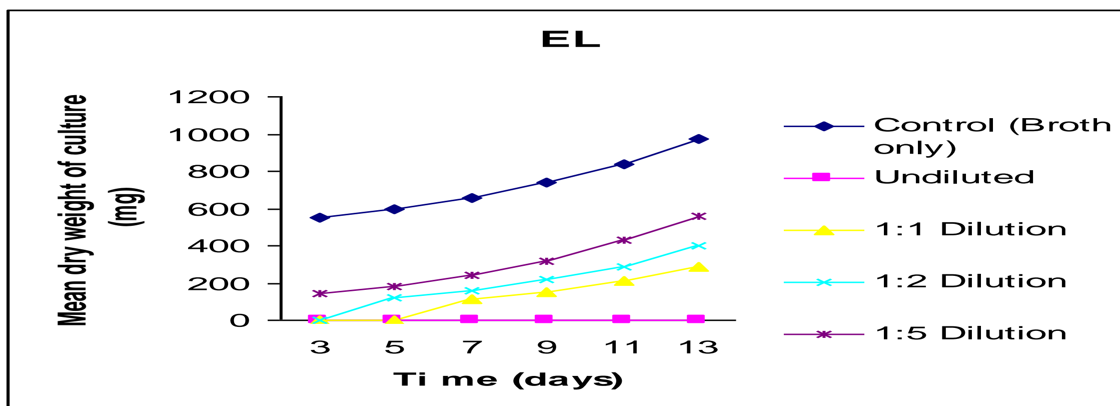


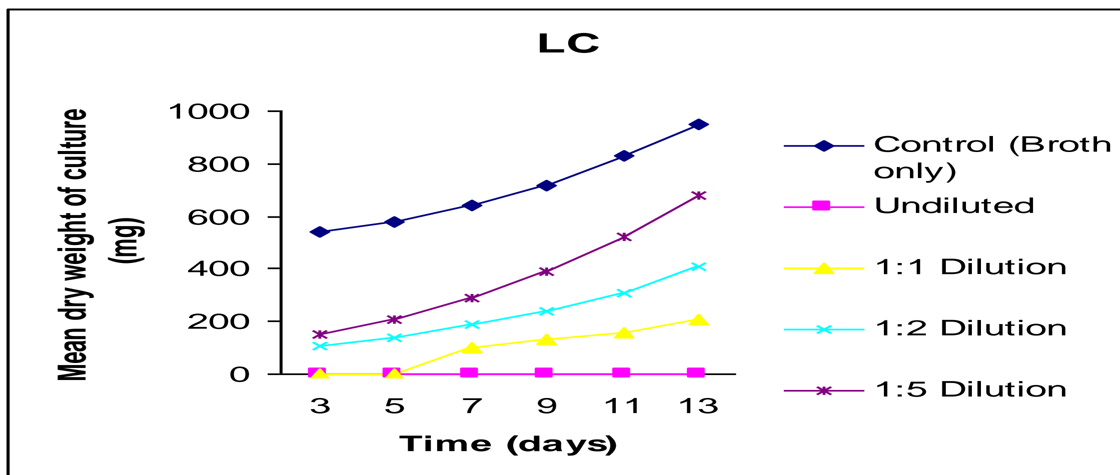


**Fig. 31.** Effect of ethanol leaf extract of cocoyam in PDB on vegetative growth of the three isolates of *S. rolfsii* at 28℃.

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Plate 3: Vegetative growth of the mycelia of the Aburi isolate of *S. rolfsii* in PDB amended with varying dilutions of ethanol leaf extract of cocoyam after 13 days $(x^{1}/_{3})$

FROM LEFT TO RIGHT: extract only, undiluted, 1:1, 1:2, 1:5 dilutions.

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## 14: STUDIES ON EFFECT OF HEAT-STERILIZED AQUEOUS LEAF EXTRACT OF PLANTAIN ON THE VEGETATIVE GROWTH OF MYCELIA OF THE THREE ISOLATES OF *SCLEROTIUM ROLFSII*.

Clearly, the phytotoxins present in the aqueous leaf extract of plantain were less toxic to the various isolates AB, EL and LC of *Sclerotium rolfsii* as compared to the ethanol leaf extract of plantain and that the results produced contrasts sharply with that of the ethanol leaf extract of plantain.. There was mycelium development for all the dilution levels and with increasing dilution, the rate of growth of the mycelia increased with greater mycelial growth occurring in 1:5 v/v dilution level for all the isolates (Fig. 38).

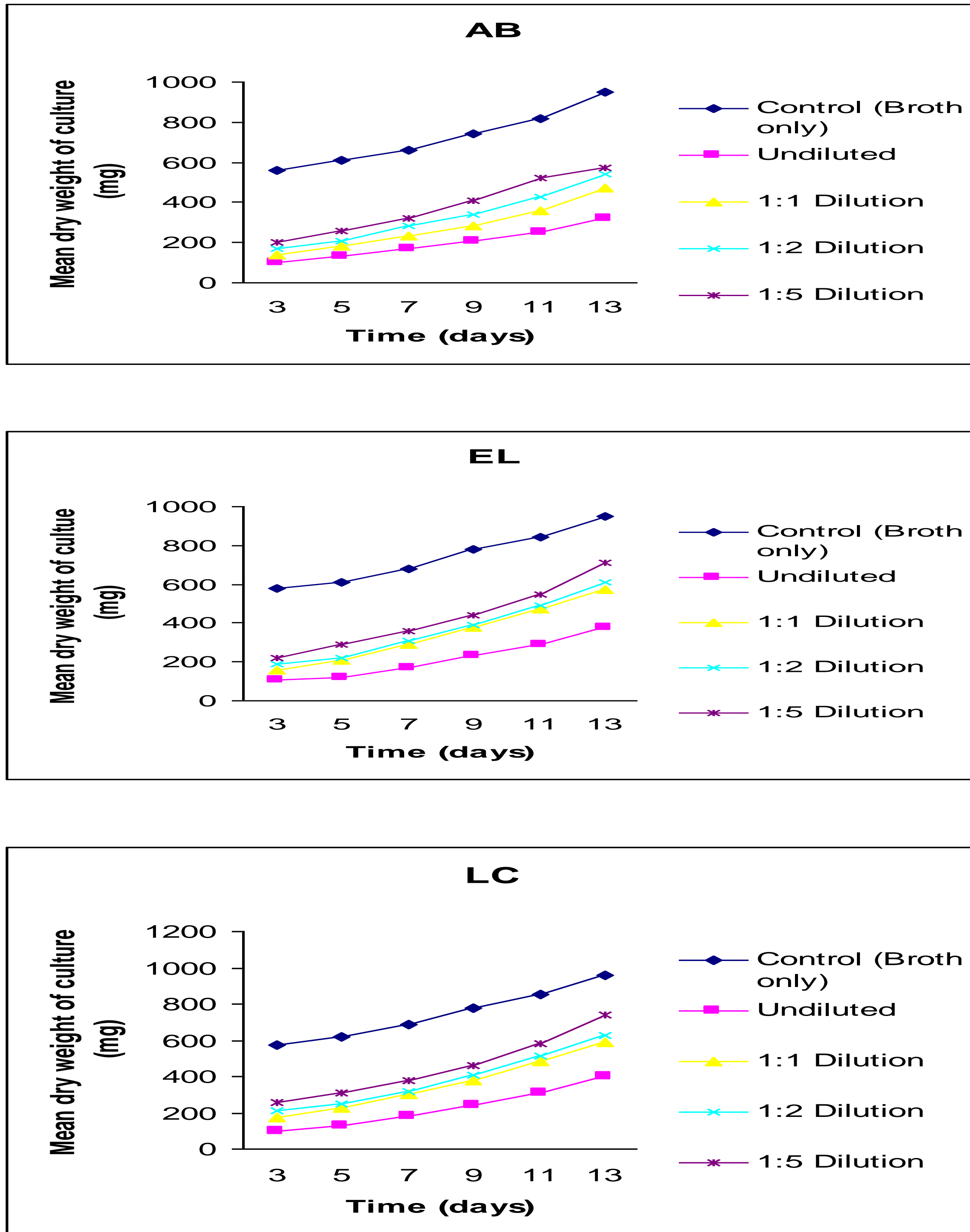


**Fig. 32**. Effect of aqueous leaf extract of plantain in PDB on vegetative growth of the three isolates of *S.rolfsii* at 28ºC.

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## 15: STUDIES ON EFFECT OF HEAT-STERILIZED AQUEOUS LEAF EXTRACT OF CASAVA ON THE VEGETATIVE GROWTH OF MYCELIA OF THE THREE ISOLATES OF *SCLEROTIUM ROLFSII*.

Just as with the heat-sterilized plantain leaf extract, the inhibition of the vegetative growth of each isolate was commensurate with the concentration of the heat-treated extract. As usual, the depression in vegetative growth was highest in the undiluted extract and the inhibitory effect of the heat-sterilized extract was gradually depressed with increasing dilution of the extract. After the maximum duration of the study, the mycelia of the AB isolate exhibited much reduced growth as compared to mycelia from the EL and LE isolates (Fig. 39).

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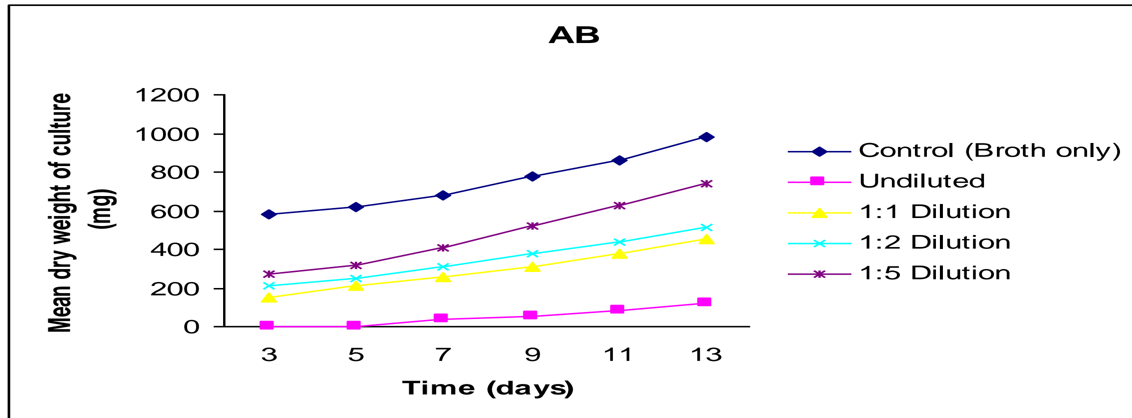


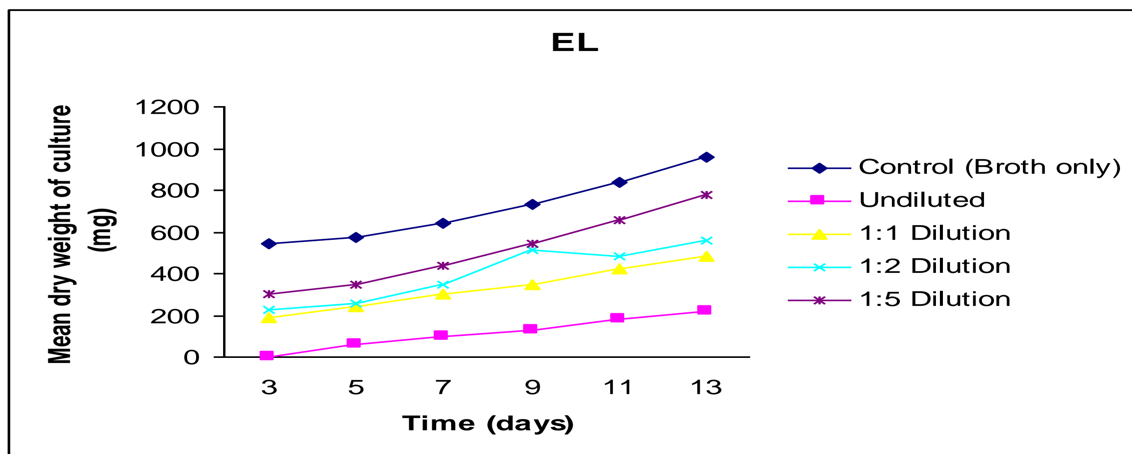


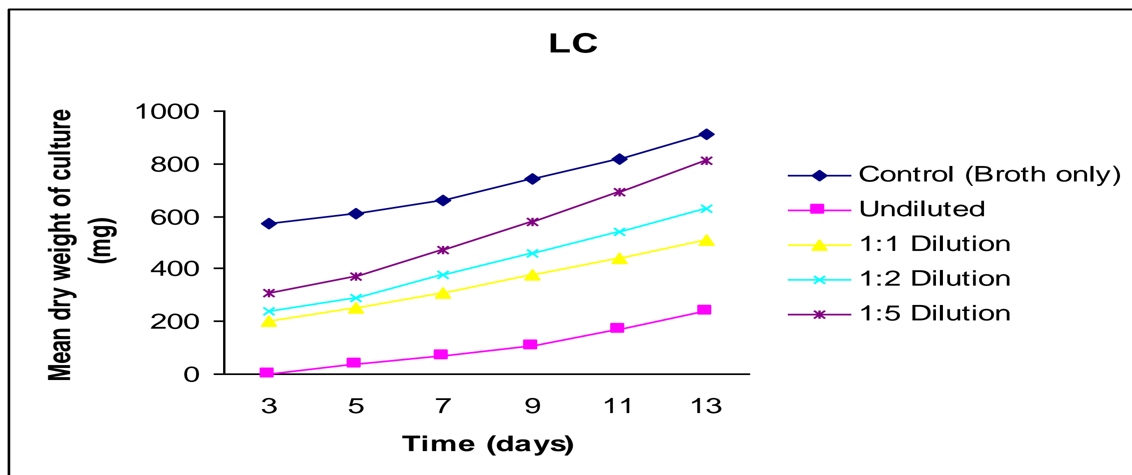


**Fig. 33**. Effect of aqueous leaf extract of cassava in PDB on vegetative growth of the three isolates of *S.rolfsii* at 28ºC.

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## 16: STUDIES ON EFFECT OF HEAT-STERILIZED AQUEOUS LEAF EXTRACT OF COCOYAM ON THE VEGETATIVE GROWTH OF MYCELIA OF THE THREE ISOLATES OF *SCLEROTIUM ROLFSII*.

Comparatively the inhibitory effect of the phytotoxins present in the heat-sterilized leaf extract of cocoyam was much potent as compared to that of plantain and cassava. The undiluted extract produced very minimal growth due to the dry potent nature of the extract and just as for the extracts of plantain and cassava, the inhibitory effect of the heat-sterilized extract was reduced with increasing dilution levels (Fig. 40).

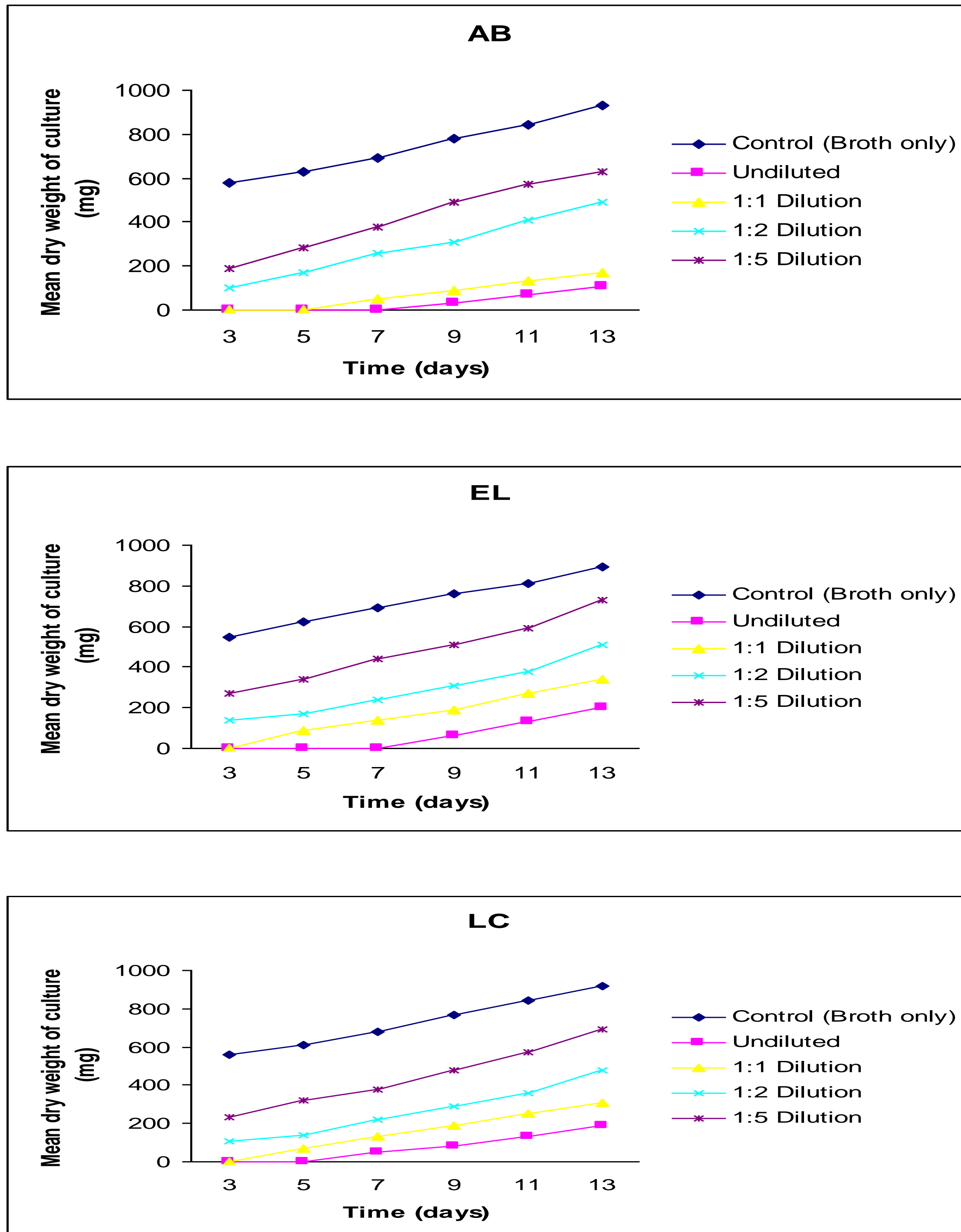


**Fig. 34**. Effect of aqueous leaf extract of cocoyam in PDB on vegetative growth of the three isolates of *S.rolfsii* at 28ºC.

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# CHAPTER FIVE

## 5.0 GENERAL DISCUSSION

Cocoyam (*Xanthosoma sagittifolium* L) is used as a source of food in many countries in the West African sub-region to augment yams and cassava which serve as the main sources of carbohydrates. In most cases cultivation of the crop is done on a small scale in Asia, Africa and Latin America since they are grown for local consumption. Cocoyam is a herbaceous monocotyledonous crop consisting basically of a corm from which offshoots such as cormels develop. It is a perennial crop whose growth cycle starts with the sprouting of leaves and the emergence of cormels from the corms. Cocoyam has been an important root crop cultivated worldwide and in addition to the cormels, the leaves also serves as an important source of carbohydrate. The final stages of the development of the cocoyam is characterized by the wilting of leaves and a decrease in dry weight above the soil level. In other instances it is used as flour due to the fact that it is highly digestible and serves as an ingredient in some baby foods. Diseases and pests of cocoyam in storage and on the farm pose a very serious problem in the production and utilization of cocoyam. During storage, losses attributed to the rotting of cormels and corms are very imminent, thus affecting both the quantity and quality of corms for planting and cormels for consumption.

*Sclerotium rolfsii* Sacc. is a common pathogen on cocoyam. The fungus is a soil-borne plant pathogen of worldwide occurrence infecting more than 500 plant species (Aycock,

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1966; Punja, 1985). It infects several species of monocotyledonous and dicotyledonous plants growing in humid areas such as tropical and sub-tropical regions and persists by the formation of sclerotia as well as the profuse growth rate of its mycelia. Some years ago, Nakata (1995), identified *Sclerotium rolfsii* to comprise of a group of species consisting of numerous biological forms and that these species can be distinguished by the phenomenon of aversion which is basically the mutual inhibition of mycelial growth by the growing colonies of different strains of the same fungal species. This phenomenon of aversion or barrage has been linked to Coyen in 1923 and since then it has been investigated as the subject for the cytoplasmic incompatibity of fused hyphae among different strains of fungi. Basically the phenomenon of aversion has been used to differentiate strains whose morphological features are quite similar to each other and it has been found to be due to the production of antibiotics which were extractable with organic solvents and their selective inhibition of the growth of the opposite strain. Aversion factors as investigated by several workers are assumed to be gaseous volatile compounds. It must however be noted that aversion occurs between different strains of the same fungus but not between two cultures of the same strain. Under normal circumstances when two colonies of the same strain are inoculated at opposite sides of an agar such as PDA in a Petri dish, they grow towards each other and meet with sclerotia being produced at the meeting zone as a result of obstruction and extensional growth of mycelia. In the same way when two colonies of different strains are inoculated at opposite sides of an agar such as PDA contained in a Petri dish, the colonies never meet and their advancing edges comes to a stop as they get near to each other. The advancing edges become separated by a distinct inhibition zone thus each

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colony forms its own distinct row of sclerotia along the boundary thus two rows of sclerotia are produced separated by the zone of inhibition.

This phenomenon of aversion has been used by Tortoe (1997) to show that several isolates of *Sclerotium rolfsii* from different products in the Greater Accra region of Ghana could be recognized as true strains because these show remarkable variation in their pre-penetration habits, pathogenicity, production of sclerotia on host tissues and the rate of degradation of host tissues. In this investigation aversion was used to distinguish between the three isolates which were isolated from naturally infested cocoyam obtained from three localities in Ghana viz Aburi, East Legon and Legon Campus. Infection produced by mycelia from a germinating sclerotia of *Sclerotium rolfsii* in the soil is dictated by whether the developing mycelia is eruptive or hyphal as well as the presence of volatile compounds, soluble nutrients and volatile compounds acting together (Punja and Grogan, 1981; Punja *et al*, 1984). The ability of an eruptively germinating sclerotia to cause an infection on a host is referred to as the competence distance and this is independent of exogenous supply of nutrients. The competence distance is a function of the fungal isolate, soil moisture, soil texture and soil depth (Punja, 1985). If they are located within the competence distance, an eruptively germinating sclerotia do not need a exogenous supply of food probably of non-living organic matter (Punja, 1985; Punja and Grogan, 1981). The initial mycelial contact with the host surface and the subsequent death of the host tissue involves a sequence of events (Bateman and Beer, 1965; Smith *et al*, 1984; Punja *et al*, 1985). Death of the host tissue caused by an eruptively germinating sclerotium is basically due to the production of large quantities of oxalic acid and polygalacturonases (Bateman, 1972) acting together and

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that this occurs prior to hyphal penetration. The aggregation of mycelia on the host's surface produces these substances that aids penetration into the host tissues through the exertion of physical pressure (Punja and Jenkins, 1984; Smith *et al*. 1984).

The function of oxalic acid in the maceration of the host's tissues is that it sequesters calcium oxalate (Punja and Jenkins, 1984) and produces a lower pH for maximum activity of endopolygalacturonases and cellulases (Bateman and Beer, 1965; Bateman, 1969, 1970, 1972; Punja *et al*. 1979). Oxalic acid itself has been found to be directly toxic to the plant tissues (Bateman and Beer, 1965). Cellulases however play a secondary role in the destruction of tissues and subsequent disease development because they appear later in the sequence of enzymes which are produced temporarily (Bateman, 1969; Sadana *et al*. 1979; Punja *et al*. 1985). The invasion of the host's tissues by hyphae is both intracellularly and intercellularly and it has been identified that hyphae contains microbodies that synthesizes calcium oxalate (Armentrout *et al.* 1978) as well as vacuoles which contains phosphotase (Hanseler *et al*. 1975; Hanseler *et al*. 1978). Advancing hyphae depletes pectic substances in the host's cells (Bateman, 1970) resulting in the production or release of peroxidase through the activity of the enzyme (Barnet, 1974). In effect the host tissues becomes water-soaked, necrotic and macerated which are characteristics of infection by the fungus *Sclerotium rolfsii*. In summary the secretion of oxalic acid and endopoygalacturonases acting together in addition to rapid mycelial growth accounts for the establishment of an infection and that differences in the endopolygalacturonase levels as well as the rates of mycelial growth are highly correlated with differences in the virulence rate of the fungus (Punja *et al*. 1985).

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Morphological characteristics of the three different isolates of *Sclerotium rolfsii* grown on PDA revealed a different mycelial growth rate for each of the isolates at 28ºC. The mycelial radial growth of each of the strains of *Sclerotium rolfsii* varied from the 3$^{rd}$ day to the 5$^{th}$ day. From the 6$^{th}$day onwards, the mycelia of all the isolates attained their maximum growth. The mean radial diameter of the three isolates AB, EL and LC differed significantly at 5% (Appendix 1). Growth and branching of filamentous *Sclerotium rolfsii* occurred at the apex of the host plant (Barnet, 1968). Growth of mycelia of *Sclerotium rolfsii* is regulated by a balance between the synthesis of cell wall and degradation of the cell wall via two enzymes namely β 1-3 glucanase and glucane synthetase. Hyphal growth and branching is controlled by an equilibrium being attained in the activities of these two enzymes (Kritzman *et al*. 1978). The formation of sclerotia were assessed after 14 days of incubation at 28ºC. Initially they appeared white and went through various colour changes such as off-white, light brown and finally to dark brown during maturity. The change in colour of sclerotia was basically due to utilization and exhaustion of nutrients. The AB isolate of *Sclerotium rolfsii* recorded the least mean diameter as well as the mean number of sclerotia formed whilst the EL isolate had the highest mean diameter with greatest number of sclerotia formed by the LC isolate. The colour and arrangement of sclerotia on PDA plates also varied for the different strains of *Sclerotium rolfsii*.

The sclerotia of the of the LC strain were arranged in zones on the PDA plate, they were initially white coloured but transformed into light brown and then dark brown. In the case of the EL isolate, white sclerotia which later changed into light brown and then dark brown formed as a ring in the center of the PDA plates, whereas for the AB strain or isolate, the

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white sclerotia that developed into light brown and then dark brown were scattered throughout the PDA plates. In the investigation it was observed that the sclerotia of all the three isolates AB, EL and LC were round in shape contrasting with other reports that suggests the production of small, spherical, tan dark brown as well as black sclerotia being produced by other strains of the fungus (Zarani and Christias, 1997; Serma *et al*. 2002). Another observation made was that the strain with greater mycelia growth, LC produced the greatest number of sclerotia on PDA in conformity with earlier investigations by Wheeler and Sharan (1963) and Zoberi (1980) that PDA that supports extensive growth also produced a greater number of sclerotia. The sclerotia of all the isolates had a shiny appearance due to the presence of a gummy material present on their surfaces. The gummy material is attributed to the production of extracelleluar polysaccharides by the strains. Factors responsible for sclerotia formation are nutritional and non-nutritional (Trevethick and Cook, 1971), depletion of nutrients (Hadar *et al*. 1983), restriction of hyphal growth due to the imposition of a physical barrier (Wheeler and Waller, 1965) and lastly depletion in nitrogen levels resulting from active growth of the fungus (Punja, 1986). The mean diameter of sclerotia and the total number of sclerotia for the three isolates (See Figs 2 and 3) differed significantly at 5% (Appendices 2 and 3).

Another observation made was that if a contaminant be it a different fungus or bacterium comes into contact with *Sclerotium rolfsii*, there was the development of more sclerotia around the contaminant. During aversion when the different strains AB, EL and LC were inoculated on the same PDA in a Petri dish, the observation made was that, the mycelia of two incompatible strains intertwined or intermingled and at later stages, the mycelia breaks

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up leading to the development of clear zone referred to as the barrage or aversion zone at the junction where the mycelia of the different strains are in contact with another. The clear zone occurred between the junctions of EL and LC, EL and AB as well as AB and LC isolates. Sclerotia were observed along the sides of the aversion zone with LC isolate producing the greatest number of sclerotia and the AB isolate the least. The total number of sclerotia produced by each isolate during aversion differed significantly at 5% (Appendix 4). This phenomenon of aversion based on mycelia compatibility and incompatibility has been used to distinguish separate strains of the same species based on morphological characteristics (Tortoe, 1997; Punja and Sun, 1997; Sarna *et al*. 2002). Further studies are highly recommended in future research as far as molecular characterization is concerned.

Microbial rotting of cocoyam cormels and corms has been a serious problem in cocoyam production and utilization. During storage, losses attributed to rotting of cormels have adverse effects in terms of quality and quantity and it has been estimated that there has been a 11% drop in production attributed to infection by *Sclerotium rolfsii* (FAO Food, 2001). The rot resulting from bottom inoculation of the cormels was suppressed in the white cormels than in the red cormels and that there was a significant difference at 5% (Appendices 5 and 6) in the mean diameter of the rots produced by the various isolates for the red and white cormels. Likewise the same trend was obtained for side inoculation of the cormels with the various isolates and that there was a significant difference at 5% in the mean diameters of the red and white cormels by the three isolates during side inoculation (Appendices 7 and 8). The effects of the development of the upper vegetative parts of the

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white and red strains of cocoyam plants as influenced by *Sclerotium rolfsii* revealed that the white strains of cocoyam were more resistant to infection by the fungus than the red strains. The development of the leaf lamina (length and width) was suppressed more in the red strain than the white strain and that there was a significant difference at 5% by the three isolates (Appendices 9, 10, 13 and 14). Other aspects such as the circumference of the stem, circumference of the petiole and the length of the petiole were suppressed in terms of development in the red strain than in the white strain and that the results produced for the various isolates differed significantly at 5% (Appendices 11, 12, 15 and 16). The infection by *Sclerotium rolfsii* resulted in arrested development of underground parts such as the corms and cormels as well. Due to the suppression in growth of the red strain than the white strain, the dry weight of the pseudostem of the white strain was more than that of the red strain for the results produced by the various isolates and that the results were significantly different at 5% for the red and white strains (Appendices 17 and 18). The dry weight of the corm of the red strain was more suppressed than the white strain for the various isolates and that the results produced for the various isolates were significantly different at 5% (Appendices 19 and 20). Infection by each of the isolates of *Sclerotium rolfsii* on the white and red types of cocoyam led to a reduction in the number of cormels and that the results produced by the various isolates were significantly different at 5% (Appendices 21 and 22).

Several reports have been made to the effect that plants that have fungicidal properties are very effective in the inhibition of fungal growth of specific fungi. These plants are known to have high levels of anthraquinones, crysophanic, napthoquonine acids capable of

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arresting fungal growth and development. In studies involving the control of *Sclerotium rolfsii* using extracts of plants, the existence of different strains were related to the inherent genetic variation of the fungus. In Experiment 5 for example, the radial growth of mycelia of the three isolates on PDA amended with ethanol leaf extracts of plantain was depressed initially by higher concentrations such as the undiluted concentration and the 1:1v/v concentration. Radial growth of all the three isolates in the higher concentrations did not in any way approximate that of the control basically because the phenolic compounds and alkaloids that constitutes the phytotoxins were actively present. On the other hand, lower concentrations such as 1:2v/v and 1:5v/v had a minimal amount of the phytotoxins present comparativel. The differences in responses of the isolates to the extracts was coupled with the inherent genetic variation of the fungus as far as the physiology of the fungus is concerned. The results produced by the AB, EL and LC isolates of *Sclerotium rolfsii* for the various dilution ratios differed significantly at 5% (Appendices 23, 35 and 42). Likewise for the ethanol leaf extracts of cassava in Experiment 6 radial growth of mycelia were depressed initially by higher concentrations such as the undiluted concentration and 1:1 v/v concentration with lessening of the depression for the lower concentrations of 1:2 and 1:5 v/v. The results produced by the three isolates of the fungus for the various dilution ratios were significantly different at 5% (Appendices 24, 36 and 48).

In the same way in Experiment 7, the radial growth of mycelia of the three isolates in response to ethanol leaf extract of cocoyam was depressed initially by higher concentrations with a reduction in the depression for lower concentrations of the extract and that the results produced by the various isolates in response to the ethanol extracts of

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cocoyam were significantly different at 5% (Appendices 25, 37 and 49). It has been reported that plants with fungicidal properties are very effective in inhibiting fungal growth *in vivo* and *in vitro* (Kuhn and Hargreaves, 1987) and in Experiments 8, 9 and 10, the radial growth of the three isolates on PDA amended with aqueous leaf extracts of plantain, cassava and cocoyam were initially depressed just as for the ethanol leaf extracts for the higher concentrations of undiluted and 1:1v/v concentrations. The ethanol leaf extract recorded higher growth inhibition than the aqueous leaf extracts. This suggests that water used in the extraction process was not able to dissolve all the active compounds present in the leaves. However the rate of inhibition of radial growth by the aqueous (water) extracts as compared to the ethanol leaf extracts was less due to the fact that the quality and quantity of the volatile phytotoxins in the leaves were less inhibitory and that the results produced by the various isolates in response to the heat-sterilized aqueous (water) extracts of plantain, cassava and cocoyam for the various dilution ratios differed significantly at 5% (Appendices 26, 27, 28, 38, 39, 40, 51, 52 and 53). On PDA, the radial growth of fungi is basically thought to be similar to what pertains in the natural environment thus the trend of depression observed for the various isolates could be likened to what actually pertains in the natural fields or environment. Though the heat-sterilized ethanol leaf extracts tend to be more potent than the aqueous leaf extracts, it was very clear that the various isolates showed differing resistance to the phytotoxins present.. Incidentally the most depression in radial growth was produced by the cocoyam ethanol leaf extracts with AB isolate being most depressed followed by the EL and LC isolates respectively. Fungistasis is defined as the inhibitory effect of chemicals, soils as well as other factors on the growth and development of fungi. In most cases, the inhibitory factors varies and its properties

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becomes lost when heat sterilized and in the presence of energy-rich material nutrients (Baker and Cook, 1974). Data obtained from this research into heat-sterilized ethanol and aqueous leaf extracts of plantain, cassava and cocoyam have revealed that heat sterilization alone does not completely destroy the phytotoxins present and that they still remain active though to a lesser extent.

There were as well very important observations made with regards to vegetative growth and sclerotium production of the three isolates in response to the heat-sterilized ethanol and aqueous leaf extracts of plantain, cassava and cocoyam. The repressive effect of the heat-sterilized leaf extracts of plantain, cassava and cocoyam on the vegetative growth of mycelia in Experiments 11, 12 and 13 gives an indication that the growth of mycelia is impaired in amended liquid cultures. The repressive effect of the undiluted concentration was almost the same for all the isolates as far as heat-sterilized ethanol leaf extracts of plantain, cassava and cocoyam were concerned as there were basically no growth in all the isolates except for a minimal growth in the LC isolate in response to the ethanol leaf extract of cassava (see Fig. 31). The results produced by the various isolates in response to ethanol leaf extracts of plantain, cassava and cocoyam for the various dilution ratios differed significantly at 5% (Appendices 29, 30, 31, 41, 42, 43, 53, 54 and 55). In comparison there was a reduction in the repressive effect of the aqueous leaf extracts of plantain, cassava and cocoyam as some amount of vegetative growth, however limited growth were recorded for the undiluted extract for all the isolates (see Figs. 34, 35 and 36). However the results produced by the various isolates in experiments 14. 15 and 16 in response to heat-sterilized aqueous leaf extract of plantain, cassava and cocoyam differed significantly for the dilution

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ratios at 5% Appendices (32, 33, 34, 44, 45, 46, 56, 57 and 58). Dilution of the heat-sterilized ethanol and aqueous leaf extracts however did reduce the potency of the biotoxins especially in the aqueous leaf extract. This implies that the phytotoxins present in the heat-sterilized ethanol leaf extracts were more potent than that present in the aqueous leaf extracts in liquid medium thus giving the indication that heat-sterilized ethanol leaf extract could be more useful as a control agent for *Sclerotium rolfsii* due to the least amount of mycelia growth and dry matter accumulation.

This agrees with earlier reports by some researchers that ethanol is a better extractant than water (Agatemor, 2009) suggesting a high proportion of ethanol soluble compounds in the leaves. As regards to the production of sclerotia by the various extracts, it became very obvious that the same trend as in dry weight of mycelia was produced. The sclerotia produced by the undiluted heat-sterilized ethanol leaf extracts of plantain, cassava and cocoyam on PDA were fewer giving the indication that the heat-sterilized ethanol leaf extract depressed sclerotium formation depending on the strain of *Sclerotium rolfsii* (see Figs 37, 38 and 39). The depression in sclerotia formation is lowered for the 1:2 v/v and 1:5 v/v concentrations as compared to the higher concentrations of undiluted and 1:1 v/v. In Experiments 17, 18 and 19, the sclerotia produced by the various isolates on PDA amended with ethanol leaf extracts of plantain, cassava and cocoyam differed significantly at 5% for the various dilution ratios (Appendices 59, 60 and 61). In comparison the heat-sterilized aqueous leaf extracts of plantain, cassava and cocoyam produced a lesser depression of sclerotium production depending on the isolate of *Sclerotium rolfsii* and that

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results produced by the various isolates differed significantly at 5% (Appendices 62, 63 and 64).

It can be inferred from this thesis that there are variations in the responses of the various isolates of *Sclerotium rolfsii* to phytotoxins present in heat-sterilized ethanol and aqueous leaf extracts of plantain, cassava and cocoyam which could be attributed to the physiological differences in the various isolates of *S.rolfsii*. This study reveals that plantain, cassava and cocoyam leaves contain fungitoxic compounds since they were able to suppress the radial and vegetative growth of the isolates of *S. rolfsii*. Further studies are recommended to identify the active components and to establish the Minimum Inhibitory Concentration (MIC) with the view of using the leaves as bioprotectants since the extracts are low cost and abundant. In addition they require simple techniques to prepare and apply and also they are very safe to handle as compared to fungicides and most importantly they leave no residues hence are environmentally friendly.

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# SUMMARY


1. Studies were carried out to study the morphological characteristics of three isolates of *Sclerotium rolfsii* from Legon campus, East Legon and Aburi localities.

2. Radial diameter of mycelia which is indicative of growth on PDA of the three isolates after 5 days of incubation was highest in the EL strain followed by the AB strain and the LC strain respectively.

3. All the three isolates of the test fungus, *Sclerotium rolfsii* attained their maximum growth after the $6^{th}$ day of incubation.

4. The EL isolate had the highest mean diameter of sclerotia followed by the LC isolate and the AB isolate in that order.

5. The LC isolate produced the greatest number of sclerotia followed by the EL and the AB isolates respectively.

6. When all the three isolates were inoculated onto the same Petri dish (aversion), the LC isolate produced the highest number of sclerotia followed by the EL and Aburi AB isolates respectively.

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7. After the maximum period of study, the mean diameter of horizontal and vertical rots produced by all the isolates were highest in the red cormels than in the white cormels.

8. There was a greater depression of the length and width of the leaf lamina in the red type of cocoyam plant than in the white type of cocoyam plant by the three isolates of *Sclerotium rolfsii* from Legon campus, East Legon and Aburi.

9. Just like the leaves of the cocoyam plants, the development of the stem of the cocoyam plant was most depressed in the red plant than in the white plant for all the three types of isolates.

10. There was a greater depression in the length and the circumference of the petiole in the red type of cocoyam plant than in the white type.

11. The dry weight of the pseudostem was highest in the white type of cocoyam plant than the red type of cocoyam plant for the respective isolates which is indicative of the fact that there was a greater depression of vegetative growth in the red cocoyam plants than the white type.

12. There was a greater depression in the development of the corm in the red cocoyam plant than the white type for all the three isolates as manifest in the dry weights of the corms after the maximum period of study.

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13. The influence of the various isolates on the development of the cormels for the red and white types of cocoyam plants were very marginal.

14. Ethanol and aqueous leaf extracts of cassava, plantain and cocoyam were used to assess their potential with respect to their ability to depress radial growth, vegetative growth and sclerotia production by the three isolates of *Sclerotium rolfsii*.

15. Radial growth of mycelia on PDA of the three isolates were initially depressed by the highest concentrations of the heat-sterilized ethanol leaf extracts of plantain, cassava and cocoyam. However after 8 days of growth, the radial growth of the LC isolate growing on media amended with heat-sterilized ethanol leaf extracts of plantain was the same as that of the control (extract-free PDA) medium for the least dilution factor of 1:5 v/v.

16. Growth of the AB isolate of *Sclerotium rolfsii*was most slowest for all the ethanol heat-sterilized leaf extracts of plantain, cassava and cocoyam tested.

17. The depression in radial growth of mycelia of all the three isolates on PDA was greater in the heat-sterilized ethanol leaf extracts than the heat-sterilized aqueous leaf extracts of plantain, cassava and cocoyam.

18. Depression of radial growth of mycelia of the three isolates of *Sclerotium rolfsii* AB, EL, and LC were highest in the undiluted ethanol and aqueous (water) leaf

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extracts of plantain, cassava and cocoyam but increasing dilutions up to 1:5 v/v gradually reduces the effect of inhibition.

19. For the heat-sterilized aqueous leaf extracts of plantain and cassava, all the three isolates achieved their maximum radial growth for the 1:5 v/v after the 8th day of incubation similar to the extract-free medium (control)

20. For the heat-sterilized aqueous leaf extracts of cocoyam however, after the 8th day of incubation for the 1:5 v/v, the AB isolate attained their maximum growth whilst the EL and LC isolates approximated that of the extract-free medium (control).

21. The depressive effect of the heat-sterilized ethanol and aqueous leaf extracts on the vegetative growth of the three isolates of *Sclerotium rolfsii*, AB, EL and LC on the vegetative growth of mycelia of the three isolates of *Sclerotium rolfsii* was highest in the extract only medium and the undiluted leaf extract, however the inhibition was gradually removed with increasing dilution (1:1-1:5 v/v) of the extract.

22. For the heat-sterilized ethanol leaf extracts of plantain, cassava and cocoyam, the extract only (control) and undiluted extract basically produced no growth of mycelia for the three isolates of *Sclerotium rolfsii*.

23. The aqueous heat-sterilized leaf extracts of plantain, cassava and cocoyam were less repressive as compared to the ethanol leaf extracts.

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24. The extract only was most repressive for all the three isolates, basically producing no mycelia growth from the $3^{rd}$ -$5^{th}$ days for the EL and LC isolates and from the $3^{rd}$ day to the $9^{th}$ day for the AB isolate and very limited growth for all the isolates thereof.

25. The heat-sterilized aqueous leaf extracts of cocoyam was more repressive on mycelia growth of the three isolates as compared to the plantain and cassava extracts.

26. The heat-sterilized aqueous leaf extracts of plantain, cassava and cocoyam were much repressive on the AB isolate of *Sclerotium rolfsii* than the other two isolates EL and LC..

27. Both the heat-sterilized aqueous and ethanol leaf extracts of plantain, cassava and cocoyam severely depressed sclerotia formation by the three isolates of *Sclerotium rolfsii* (AB, EL and LC), however there were various differences between the various isolates.

28. Depression in sclerotia formation by mycelia of the various isolates was greatest in the undiluted extract with the depression or inhibitory effect being removed with increasing dilutions.

29. Generally the undiluted heat-sterilized ethanol leaf extract of plantain, cassava and

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cocoyam depressed sclerotia formation by 90.0%-95.8% depending on the isolate whilst the heat-sterilized undiluted aqueous leaf extracts depressed sclerotia formation by 84%-91.9% depending on the isolate.

30. The lowest number of sclerotia for the heat-sterilized undiluted ethanol leaf extracts of plantain, cassava and cocoyam was recorded by the AB isolate for the heat-sterilized leaf extract of cocoyam whilst the highest number was recorded by the LC isolate for the heat-sterilized leaf extract of cassava.

31. For the undiluted aqueous leaf extracts of plantain, cassava and cocoyam, the lowest number of sclerotia were produced by the AB isolate in response to the heat-sterilized leaf extract of plantain whilst the highest number was recorded by the LC isolate in response to the extract of plantain.

32. Results as produced from my investigations have revealed the potential use of heat-sterilized ethanol and aqueous extracts of plantain, cassava and cocoyam to reduce radial growth of mycelia and sclerotia formation by *Sclerotium rolfsii* isolated from Legon campus, East Legon and Aburi with the ethanol extracts being the most potent.

33. Other results of the investigation also gives an indication of the influence of the three isolates of *Sclerotium rolfsii* on the cormel, corm and vegetative development of the red and white types of cocoyam with the white type being in most cases less

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susceptible to infection by *Sclerotium rolfsii* as compared to the red type.

34. The practical implications of these research findings have already been discussed and further studies are recommended to supplement these findings.

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# APPENDICES

## APPENDIX 1

Variate: Mean radial diameter of culture

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.00250 | 0.00250 | 0.20 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 194.83139 | 38.96628 | 3117.30 | <0.001 |
| Isolates | 2 | 0.48389 | 0.24194 | 19.36 | <0.001 |
| Days.Isolates | 10 | 2.76611 | 0.27661 | 22.13 | <0.001 |
| Residual | 17 | 0.21250 | 0.01250 | | |
| Total | 35 | 198.29639 | | | |

## APPENDIX 2

Variate: Mean diameter of sclerotia

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.06000 | 0.06000 | 1.33 | |
| Rep.*Units* stratum | | | | | |
| Isolates | 2 | 1.21000 | 0.60500 | 13.44 | <.001 |
| Residual | 2 | 0.09000 | 0.04500 | | |
| Total | 5 | 1.36000 | | | |

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**APPENDIX 3**

Variate: Total number of sclerotia

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 8.817 | 8.817 | 27.84 | |
| Isolates | 2 | 1.803 | 9.015 | 2.847 | <.001 |
| Residual | 2 | 6.333 | 3.167 | | |
| Total | 5 | 1.803 | | | |

**APPENDIX 4**

Variate: Total number of sclerotia using the phenomenon of aversion.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.32667 | 0.32667 | 10.32 | |
| Isolates | 2 | 8320.06333 | 4160.03167 | 1314.05 | <.001 |
| Residual | 2 | 0.06333 | 0.03167 | | |
| Total | 5 | 8320.45333 | | | |

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**APPENDIX 5**

Variate: Mean diameter of rot of bottom inoculation of the red type of cormel

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.03361 | 0.03361 | 0.64 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 949.13139 | 189.82628 | 3620.25 | <.001 |
| Isolates | 2 | 23.50389 | 11.75194 | 224.13 | <.001 |
| Days.Isolates | 10 | 11.68278 | 1.16828 | 22.28 | <.001 |
| Residual | 17 | 0.89139 | 0.05243 | | |
| Total | 35 | 985.24306 | | | |

**APPENDIX 6**

Variate: Mean diameter of rot of bottom inoculation of the white type of cormel

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.10028 | 0.10028 | 4.43 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 519.53139 | 103.90628 | 4591.38 | <.001 |
| Isolates | 2 | 51.58222 | 25.79111 | 1139.65 | <.001 |
| Days.Isolates | 10 | 41.77778 | 4.17778 | 184.61 | <.001 |
| Residual | 17 | 0.38472 | 0.02263 | | |
| Total | 35 | 613.37639 | | | |

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**APPENDIX 7**

Variate: Mean diameter of rot of side inoculation of the white type of cormel

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.05444 | 0.05444 | 4.10 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 620.96889 | 124.19378 | 9360.42 | <.001 |
| Isolates | 2 | 18.28722 | 9.14361 | 689.15 | <.001 |
| Days.Isolates | 10 | 9.24278 | 0.92428 | 69.66 | <.001 |
| Residual | 17 | 0.22556 | 0.01327 | | |
| Total | 35 | 648.77889 | | | |

**APPENDIX 8**

Variate: Mean diameter of rot of side inoculation of the red type of cormel

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.233611 | 0.233611 | 43.46 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 768.931389 | 153.786278 | 28607.05 | <.001 |
| Isolates | 2 | 0.180556 | 0.090278 | 16.79 | <.001 |
| Days.Isolates | 10 | 13.572778 | 1.357278 | 252.48 | <.001 |
| Residual | 17 | 0.091389 | 0.005376 | | |
| Total | 35 | 783.009722 | | | |

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## APPENDIX 9

Variate: Mean length of leaf lamina of the white type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.20021 | 0.20021 | 17.39 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 142.00854 | 28.40171 | 2466.99 | <.001 |
| Isolates | 3 | 25.92562 | 8.64187 | 750.64 | <.001 |
| Days.Isolates | 15 | 81.28062 | 5.41871 | 470.67 | <.001 |
| Residual | 23 | 0.26479 | 0.01151 | | |
| Total | 47 | 249.67979 | | | |

## APPENDIX 10

Variate: Mean width of leaf lamina of the white type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.35021 | 0.35021 | 23.36 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 38.75104 | 7.75021 | 516.99 | <.001 |
| Isolates | 3 | 7.76229 | 2.58743 | 172.60 | <.001 |
| Days.Isolates | 15 | 3.53146 | 0.23543 | 15.70 | <.001 |
| Residual | 23 | 0.34479 | 0.01499 | | |
| Total | 47 | 50.73979 | | | |

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## APPENDIX 11

Variate: Mean circumference of the stem of the white type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.016875 | 0.016875 | 2.06 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 13.978542 | 2.795708 | 341.80 | <.001 |
| Isolates | 3 | 19.220625 | 6.406875 | 783.30 | <.001 |
| Days.Isolates | 15 | 2.235625 | 0.149042 | 18.22 | <.001 |
| Residual | 23 | 0.188125 | 0.008179 | | |
| Total | 47 | 35.639792 | | | |

## APPENDIX 12

Variate: Mean length of the petiole of the white type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.12000 | 0.12000 | 6.27 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 31.34417 | 6.26883 | 327.69 | <.001 |
| Isolates | 3 | 19.79083 | 6.59694 | 344.84 | <.001 |
| Days.Isolates | 15 | 4.32417 | 0.28828 | 15.07 | <.001 |
| Residual | 23 | 0.44000 | 0.01913 | | |
| Total | 47 | 56.01917 | | | |

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## APPENDIX 13

Variate: Mean circumference of the petiole of the white type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.025208 | 0.025208 | 2.76 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 2.283542 | 0.456708 | 50.07 | <.001 |
| Isolates | 3 | 6.425625 | 2.141875 | 234.82 | <.001 |
| Days.Isolates | 15 | 0.510625 | 0.034042 | 3.73 | <.001 |
| Residual | 23 | 0.209792 | 0.009121 | | |
| Total | 47 | 9.454792 | | | |

## APPENDIX 14

Variate: Mean length of the leaf lamina of the red type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.06750 | 0.06750 | 3.29 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 48.92167 | 9.78433 | 476.27 | <.001 |
| Isolates | 3 | 32.73417 | 10.91139 | 531.14 | <.001 |
| Days.Isolates | 15 | 4.10333 | 0.27356 | 13.32 | <.001 |
| Residual | 23 | 0.47250 | 0.02054 | | |
| Total | 47 | 86.29917 | | | |

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## APPENDIX 15

Variate: Mean width of leaf lamina of the red type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.09188 | 0.09188 | 4.29 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 50.14854 | 10.02971 | 467.80 | <.001 |
| Isolates | 3 | 59.56896 | 19.85632 | 926.12 | <.001 |
| Days.Isolates | 15 | 3.57729 | 0.23849 | 11.12 | <.001 |
| Residual | 23 | 0.49313 | 0.02144 | | |
| Total | 47 | 113.87979 | | | |

## APPENDIX 16

Variate: Mean circumference of the stem of the red type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.22688 | 0.22688 | 3.84 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 46.23354 | 9.24671 | 156.59 | <.001 |
| Isolates | 3 | 55.06229 | 18.35410 | 310.83 | <.001 |
| Days.Isolates | 15 | 4.57396 | 0.30493 | 5.16 | <.001 |
| Residual | 23 | 1.35812 | 0.05905 | | |
| Total | 47 | 107.45479 | | | |

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## APPENDIX 17

Variate: Mean length of the petiole of the red type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 13380. | 13380. | 1.00 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 68604. | 13721. | 1.03 | < .001 |
| Isolates | 3 | 38284. | 12761. | 0.96 | < .001 |
| Days.Isolates | 15 | 200082. | 13339. | 1.00 | < .001 |
| Residual | 23 | 306781. | 13338. | | |
| Total | 47 | 627131. | | | |

## APPENDIX 18

Variate: Mean circumference of the petiole of the red type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.12000 | 0.12000 | 4.52 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 4.68167 | 0.93633 | 35.30 | <.001 |
| Isolates | 3 | 6.08250 | 2.02750 | 76.45 | <.001 |
| Days.Isolates | 15 | 0.72500 | 0.04833 | 1.82 | <.001 |
| Residual | 23 | 0.61000 | 0.02652 | | |
| Total | 47 | 12.21917 | | | |

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## APPENDIX 19

Variate: Mean dry weight of the upper vegetative parts of the white type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.000000 | 0.000000 | 0.00 | |
| Rep.*Units* stratum | | | | | |
| Isolates | 3 | 12.335000 | 4.111667 | 616.75 | <.001 |
| Residual | 3 | 0.020000 | 0.006667 | | |
| Total | 7 | 12.355000 | | | |

## APPENDIX 20

Variate: Mean dry weight of the upper vegetative parts of the red type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.01125 | 0.01125 | 0.40 | |
| Rep.*Units* stratum | | | | | |
| Isolates | 3 | 52.68375 | 17.56125 | 629.06 | <.001 |
| Residual | 3 | 0.08375 | 0.02792 | | |
| Total | 7 | 52.77875 | | | |

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**APPENDIX 21**

Variate: Mean dry weight of the corm of the white type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.012800 | 0.012800 | 14.00 | |
| Rep.*Units* stratum | | | | | |
| Isolates | 3 | 6.7061500 | 2.2353833 | 2794.23 | <.001 |
| Residual | 3 | 0.0024000 | 0.0008000 | | |
| Total | 7 | 6.7213500 | | | |

**APPENDIX 22**

Variate: Mean dry weight of the corm of the red type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.045000 | 0.045000 | 9.00 | |
| Rep.*Units* stratum | | | | | |
| Isolates | 3 | 440.360000 | 146.786667 | 29357.33 | <.001 |
| Residual | 3 | 0.015000 | 0.005000 | | |
| Total | 7 | 440.420000 | | | |

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## APPENDIX 23

Variate: Mean number of cormels of the white type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.0128000 | 0.0128000 | 16.00 | |
| Rep.*Units* stratum | | | | | |
| Isolates | 3 | 6.7061500 | 2.2353833 | 2794.23 | <.001 |
| Residual | 3 | 0.0024000 | 0.0008000 | | |
| Total | 7 | 6.7213500 | | | |

## APPENDIX 24

Variate: Mean number of cormels of the red type of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.0128000 | 0.0128000 | 16.00 | |
| Rep.*Units* stratum | | | | | |
| Isolates | 3 | 6.7061500 | 2.2353833 | 2794.23 | <.001 |
| Residual | 3 | 0.0024000 | 0.0008000 | | |
| Total | 7 | 6.7213500 | | | |

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## APPENDIX 25

Variate: Mean radial diameter of mycelia of the AB isolate in ethanol leaf extracts of plantain

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.07350 | 0.07350 | 4.72 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 20.59150 | 4.11830 | 264.52 | <.001 |
| Dilution_Ratio | 4 | 254.48933 | 63.62233 | 4086.48 | <.001 |
| Days.Dilution_Ratio | 20 | 7.08267 | 0.35413 | 22.75 | <.001 |
| Residual | 29 | 0.45150 | 0.01557 | | |
| Total | 59 | 282.68850 | | | |

## APPENDIX 26

Variate: Mean radial diameter of mycelia of the AB isolate in ethanol leaf extracts of cassava

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.05400 | 0.05400 | 1.10 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 54.55533 | 10.91107 | 221.89 | <.001 |
| Dilution_Ratio | 4 | 198.79933 | 49.69983 | 1010.73 | <.001 |
| Days.Dilution_Ratio | 20 | 4.48467 | 0.22423 | 4.56 | <.001 |
| Residual | 29 | 1.42600 | 0.04917 | | |
| Total | 59 | 259.31933 | | | |

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## APPENDIX 27

Variate: Mean radial diameter of mycelia of the AB isolate in ethanol leaf extracts of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.03750 | 0.03750 | 1.35 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 38.64883 | 7.72977 | 277.60 | <.001 |
| Dilution_Ratio | 4 | 207.09233 | 51.77308 | 1859.34 | <.001 |
| Days.Dilution_Ratio | 20 | 7.94367 | 0.39718 | 14.26 | <.001 |
| Residual | 29 | 0.80750 | 0.02784 | | |
| Total | 59 | 254.52983 | | | |

## APPENDIX 28

Variate: Mean radial diameter of mycelia of the AB isolate in aqueous (water) leaf extracts of plantain.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.24067 | 0.24067 | 11.45 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 93.70333 | 18.74067 | 891.92 | <.001 |
| Dilution_Ratio | 4 | 106.39733 | 26.59933 | 1265.94 | <.001 |
| Days.Dilution_Ratio | 20 | 14.58667 | 0.72933 | 34.71 | <.001 |
| Residual | 29 | 0.60933 | 0.02101 | | |
| Total | 59 | 215.53733 | | | |

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## APPENDIX 29

Variate: Mean radial diameter of mycelia of the AB isolate in aqueous (water) leaf extracts of cassava.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.09600 | 0.09600 | 5.12 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 101.68533 | 20.33707 | 1084.15 | <.001 |
| Dilution_Ratio | 4 | 117.87733 | 29.46933 | 1570.98 | <.001 |
| Days.Dilution_Ratio | 20 | 10.87467 | 0.54373 | 28.99 | <.001 |
| Residual | 29 | 0.54400 | 0.01876 | | |
| Total | 59 | 231.07733 | | | |

## APPENDIX 30

Variate: Mean radial diameter of mycelia of the AB isolate in aqueous (water) leaf extracts of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.09600 | 0.09600 | 9.17 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 113.74333 | 22.74867 | 2180.10 | <.001 |
| Dilution_Ratio | 4 | 82.27567 | 20.57142 | 1962.40 | <.001 |
| Days.Dilution_Ratio | 20 | 4.82833 | 0.22142 | 23.03 | <.001 |
| Residual | 29 | 0.30400 | 0.01148 | | |
| Total | 59 | 201.25733 | | | |

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## APPENDIX 31

Variate: Mean dry weight of culture of the AB isolate in heat-sterilized ethanol leaf extracts of plantain

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.00500 | 0.00500 | 0.33 | 0.568 |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 0.36647 | 0.07329 | 4.88 | < 0.001 |
| Dilution_Ratio | 5 | 3.80371 | 0.76074 | 50.67 | < 0.001 |
| Days.Dilution_Ratio | 25 | 0.54897 | 0.02196 | 1.46 | < 0.148 |
| Residual | 35 | 0.52551 | 0.01501 | | |
| Total | 71 | 5.24886 | 0.07393 | | |

## APPENDIX 32

Variate: Mean dry weight of the AB isolate in heat-sterilized ethanol leaf extracts of cassava

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.0025681 | 0.0025681 | 7.53 | 0.009 |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 0.4246448 | 0.0849290 | 249.09 | < 0.001 |
| Dilution_Ratio | 5 | 4.4281675 | 0.8856335 | 2597.49 | < 0.001 |
| Days.Dilution_Ratio | 25 | 0.2898226 | 0.0115929 | 34.00 | < 0.001 |
| Residual | 35 | 0.0119335 | 0.0003410 | | |
| Total | 71 | 5.1543875 | 0.0725970 | | |

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## APPENDIX 33

Variate: Mean dry weight of mycelia of the AB isolate in heat-sterilized ethanol leaf extract of cocoyam.

| Source | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep | 1 | 0.0000347 | 0.0000347 | 0.07 | 0.790 |
| Days | 5 | 0.2426063 | 0.0485213 | 100.40 | < 0.001 |
| Dilution_Ratio | 5 | 4.2727064 | 0.8545413 | 1768.23 | < 0.001 |
| Days.Dilution_Ratio | 25 | 0.1756526 | 0.0070261 | 14.54 | < 0.001 |
| Residual | 35 | 0.0169146 | 0.0004833 | | |
| Total | 71 | 4.7064319 | 0.0662878 | | |

## APPENDIX 34

Variate: Mean dry weight of culture of the AB isolate in heat-sterilized aqueous (water) leaf extracts of plantain

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.001012 | 0.001012 | 0.40 | 0.009 |
| Rep.*Units* stratum | | | | | |
| Dilution_Ratio | 5 | 2.519229 | 0.503846 | 200.54 | <.001 |
| Days | 5 | 0.964979 | 0.192996 | 76.81 | <.001 |
| Dilution_Ratio.Days | 25 | 0.221829 | 0.008873 | 3.53 | <.001 |
| Residual | 35 | 0.087938 | 0.002513 | | |
| Total | 71 | 3.794987 | | | |

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## APPENDIX 35

Variate: Mean dry weight of culture of the AB isolate in heat-sterilized aqueous (water) leaf extracts of cassava.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.0024500 | 0.0024500 | 2.76 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 0.5751944 | 0.1150389 | 129.67 | <.001 |
| Dilution_Ratio | 5 | 4.2097278 | 0.8419456 | 949.05 | <.001 |
| Days.Dilution_Ratio | 25 | 0.2489722 | 0.0099589 | 11.23 | <.001 |
| Residual | 35 | 0.0310500 | 0.0008871 | | |

## APPENDIX 36

Variate: Mean dry weight of culture of the AB isolate in heat-sterilized aqueous (water) leaf extract of cocoyam.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.006050 | 0.006050 | 2.66 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 0.350728 | 0.070146 | 30.86 | <.001 |
| Dilution_Ratio | 5 | 4.332711 | 0.866542 | 381.26 | <.001 |
| Days.Dilution_Ratio | 25 | 0.384022 | 0.015361 | 6.76 | <.001 |
| Residual | 35 | 0.079550 | 0.002273 | | |
| Total | 71 | 5.153061 | | | |

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## APPENDIX 37

Variate: Mean radial diameter of mycelia of the EL isolate in heat-sterilized ethanol leaf extract of plantain.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.28017 | 0.28017 | 5.06 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 67.01950 | 13.40390 | 242.21 | <.001 |
| Dilution_Ratio | 4 | 151.46433 | 37.86608 | 684.26 | <.001 |
| Days.Dilution_Ratio | 20 | 6.73967 | 0.33698 | 6.09 | <.001 |
| Residual | 29 | 1.60483 | 0.05534 | | |
| Total | 59 | 227.10850 | | | |

## APPENDIX 38

Variate: Mean radial diameter of mycelia of the EL strain in heat-sterilized ethanol leaf extract of cassava

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.04267 | 0.04267 | 1.46 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 48.30333 | 9.66067 | 330.64 | <.001 |
| Dilution_Ratio | 4 | 132.92233 | 33.23058 | 1137.32 | <.001 |
| Days.Dilution_Ratio | 20 | 5.29167 | 0.26458 | 9.06 | <.001 |
| Residual | 29 | 0.84733 | 0.02922 | | |
| Total | 59 | 187.40733 | | | |

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## APPENDIX 39

Variate: Mean radial diameter of mycelia of the EL isolate in heat-sterilized ethanol leaf extract of cocoyam.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.10417 | 0.10417 | 6.15 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 52.72083 | 10.54417 | 622.98 | <.001 |
| Dilution_Ratio | 4 | 209.33400 | 52.33350 | 3092.03 | <.001 |
| Days.Dilution_Ratio | 20 | 8.16000 | 0.40800 | 24.11 | <.001 |
| Residual | 29 | 0.49083 | 0.01693 | | |
| Total | 59 | 270.80983 | | | |

## APPENDIX 40

Variate: Mean radial diameter of mycelia of the EL isolate in heat-sterilized aqueous (water) leaf extract of plantain

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.11267 | 0.11267 | 3.28 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 102.68600 | 20.53720 | 597.17 | <.001 |
| Dilution_Ratio | 4 | 99.74433 | 24.93608 | 725.08 | <.001 |
| Days.Dilution_Ratio | 20 | 4.07567 | 0.20378 | 5.93 | <.001 |
| Residual | 29 | 0.99733 | 0.03439 | | |
| Total | 59 | 207.61600 | | | |

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## APPENDIX 41

Variate: Mean radial diameter of mycelia of the EL strain in aqueous (water) leaf extract of cassava

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.17067 | 0.17067 | 5.63 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 97.97200 | 19.59440 | 646.21 | <.001 |
| Dilution_Ratio | 4 | 100.93767 | 25.23442 | 832.22 | <.001 |
| Days.Dilution_Ratio | 20 | 3.20633 | 0.16032 | 5.29 | <.001 |
| Residual | 29 | 0.87933 | 0.03032 | | |
| Total | 59 | 203.16600 | | | |

## APPENDIX 42

Variate: Mean radial diameter of mycelia of the EL isolate in aqueous (water) leaf extract of cocoyam.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.09600 | 0.09600 | 9.16 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 113.74333 | 22.74867 | 2170.10 | <.001 |
| Dilution_Ratio | 4 | 82.28567 | 20.57142 | 1962.40 | <.001 |
| Days.Dilution_Ratio | 20 | 4.82833 | 0.24142 | 23.03 | <.001 |
| Residual | 29 | 0.30400 | 0.01048 | | |
| Total | 59 | 201.25733 | | | |

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## APPENDIX 43

Variate: Mean dry weight of culture of the EL isolate in heat-sterilized ethanol leaf extract of plantain.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.001513 | 0.001513 | 1.07 | 0.309 |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 0.758427 | 0.151685 | 107.02 | < 0.001 |
| Dilution_Ratio | 5 | 1.949290 | 0.389858 | 275.05 | < 0.001 |
| Days.Dilution_Ratio | 25 | 1.092247 | 0.043690 | 30.82 | < 0.001 |
| Residual | 35 | 0.049609 | 0.001417 | | |
| Total | 71 | 3.838465 | 0.054063 | | |

## APPENDIX 44

Variate: Mean dry weight of culture of the EL isolate in heat-sterilized ethanol leaf extract of cassava.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.0022222 | 0.0022222 | 2.94 | 0.095 |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 0.3947114 | 0.0789423 | 104.52 | < 0.001 |
| Dilution_Ratio | 5 | 4.4774333 | 0.8954867 | 1185.59 | < 0.001 |
| Days.Dilution_Ratio | 25 | 0.2680753 | 0.0107230 | 14.20 | < 0.001 |
| Residual | 35 | 0.0264358 | 0.0007553 | | |
| Total | 71 | 5.1662000 | 0.0727634 | | |

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## APPENDIX 45

Variate: Mean dry weight of culture of the EL isolate in heat-sterilized ethanol leaf extracts of cocoyam.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.000672 | 0.000672 | 0.42 | 0.523 |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 0.401537 | 0.080307 | 49.65 | < 0.001 |
| Dilution_Ratio | 5 | 4.407172 | 0.881434 | 544.91 | < 0.001 |
| Days.Dilution_Ratio | 25 | 0.268158 | 0.010726 | 6.63 | < 0.001 |
| Residual | 35 | 0.056615 | 0.001618 | | |
| Total | 71 | 5.130950 | 0.072267 | | |

## APPENDIX 46

Variate: Mean dry weight of culture of the EL isolate in heat-sterilized aqueous (water) leaf extract of plantain.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.0002347 | 0.0002347 | 0.75 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 1.0654236 | 0.2130847 | 683.26 | <.001 |
| Dilution_Ratio | 5 | 2.6938569 | 0.5387714 | 1727.58 | <.001 |
| Days.Dilution_Ratio | 25 | 0.1302347 | 0.0052094 | 16.70 | <.001 |
| Residual | 35 | 0.0109153 | 0.0003119 | | |
| Total | 71 | 3.9006653 | | | |

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## APPENDIX 47

Variate: Mean dry weight of culture of the EL isolate in heat-sterilized aqueous (water) leaf extract of cassava.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.003200 | 0.003200 | 2.83 | |
| Rep.*Units* stratum | | | | | |
| Dilution_Ratio | 5 | 3.381283 | 0.676257 | 597.70 | <.001 |
| Days | 5 | 0.811783 | 0.162357 | 143.50 | <.001 |
| Dilution_Ratio.Days | 25 | 0.177933 | 0.007117 | 6.29 | <.001 |
| Residual | 35 | 0.039600 | 0.001131 | | |
| Total | 71 | 4.413800 | | | |

## APPENDIX 48

Variate: Mean dry weight of culture of the EL isolate in heat-sterilized aqueous (water) leaf extract of cocoyam

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.002335 | 0.002335 | 2.31 | |
| Rep.*Units* stratum | | | | | |
| Dilution_Ratio | 5 | 4.220862 | 0.844172 | 836.64 | <.001 |
| Days | 5 | 0.614079 | 0.122816 | 121.72 | <.001 |
| Dilution_Ratio.Days | 25 | 0.180296 | 0.007212 | 7.15 | <.001 |
| Residual | 35 | 0.035315 | 0.001009 | | |
| Total | 71 | 5.052887 | | | |

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## APPENDIX 49

Variate: Mean radial diameter of mycelia of the LC isolate in heat-sterilized ethanol leaf extract of plantain.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.15000 | 0.15000 | 8.37 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 42.53133 | 8.50627 | 474.39 | <.001 |
| Dilution_Ratio | 4 | 77.19067 | 19.29767 | 1076.22 | <.001 |
| Days.Dilution_Ratio | 20 | 5.11533 | 0.25577 | 14.26 | <.001 |
| Residual | 29 | 0.52000 | 0.01793 | | |
| Total | 59 | 125.50733 | | | |

## APPENDIX 50

Variate: Mean radial diameter of mycelia of the LC isolate in heat-sterilized ethanol leaf extract of cassava.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.36817 | 0.36817 | 15.77 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 49.25750 | 9.85150 | 422.10 | <.001 |
| Dilution_Ratio | 4 | 187.13167 | 46.78292 | 2004.49 | <.001 |
| Days.Dilution_Ratio | 20 | 5.85833 | 0.29292 | 12.55 | <.001 |
| Residual | 29 | 0.67683 | 0.02334 | | |
| Total | 59 | 243.29250 | | | |

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**APPENDIX 51**

Variate: Mean radial diameter of mycelia of the LC isolate in heat-sterilized ethanol leaf extract of cocoyam.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.20417 | 0.20417 | 10.56 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 43.67483 | 8.73497 | 451.67 | <0.001 |
| Dilution_Ratio | 4 | 173.09600 | 43.27400 | 2237.65 | <0.001 |
| Days.Dilution_Ratio | 20 | 5.44600 | 0.27230 | 14.08 | <0.001 |
| Residual | 29 | 0.56083 | 0.01934 | | |
| Total | 59 | 222.98183 | | | |

**APPENDIX 52**

Variate: Mean radial diameter of mycelia of the LC isolate in heat-sterilized aqueous (water) leaf extract of plantain.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.07350 | 0.07350 | 3.00 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 89.22483 | 17.84497 | 727.34 | <.001 |
| Dilution_Ratio | 4 | 77.16333 | 19.29083 | 786.27 | <.001 |
| Days.Dilution_Ratio | 20 | 9.63267 | 0.48163 | 19.63 | <.001 |
| Residual | 29 | 0.71150 | 0.02453 | | |
| Total | 59 | 176.80583 | | | |

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## APPENDIX 53

Variate: Mean radial diameter of mycelia of the LC isolate in heat-sterilized aqueous (water) leaf extract of cassava

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.17067 | 0.17067 | 5.63 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 97.97200 | 19.59440 | 646.21 | <.001 |
| Dilution_Ratio | 4 | 100.93767 | 25.23442 | 832.22 | <.001 |
| Days.Dilution_Ratio | 20 | 3.20633 | 0.16032 | 5.29 | <.001 |
| Residual | 29 | 0.87933 | 0.03032 | | |
| Total | 59 | 203.16600 | | | |

## APPENDIX 54

Variate: Mean radial diameter of mycelia of the LC isolate in heat-sterilized aqueous (water) leaf extract of cocoyam.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.06667 | 0.06667 | 4.68 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 79.11600 | 15.82320 | 1110.18 | <.001 |
| Dilution_Ratio | 4 | 137.49767 | 34.37442 | 2411.75 | <.001 |
| Days.Dilution_Ratio | 20 | 17.28233 | 0.86412 | 60.63 | <.001 |
| Residual | 29 | 0.41333 | 0.01425 | | |
| Total | 59 | 234.37600 | | | |

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## APPENDIX 55

Variate: Mean dry weight of culture of the LC isolate in heat-sterilized ethanol leaf extract of plantain.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.0011681 | 0.0011681 | 1.51 | 0.228 |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 0.7196916 | 0.1439383 | 185.49 | < 0.001 |
| Dilution_Ratio | 5 | 4.6972943 | 0.9394589 | 1210.65 | < 0.001 |
| Days.Dilution_Ratio | 25 | 0.4355027 | 0.0174201 | 22.45 | < 0.001 |
| Residual | 35 | 0.0271599 | 0.0007760 | | |
| Total | 71 | 5.8768319 | 0.0827723 | | |

## APPENDIX 56

Variate: Mean dry weight of culture of the LC isolate in heat-sterilized ethanol leaf extract of cassava.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.0010889 | 0.0010889 | 2.91 | 0.097 |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 0.4447975 | 0.0889595 | 237.85 | < 0.001 |
| Dilution_Ratio | 5 | 4.4754833 | 0.8950967 | 2393.20 | < 0.001 |
| Days.Dilution_Ratio | 25 | 0.2554039 | 0.0102162 | 27.31 | < 0.001 |
| Residual | 35 | 0.0130906 | 0.0003740 | | |
| Total | 71 | 5.1869778 | 0.0730560 | | |

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## APPENDIX 57

Variate: Mean dry weight of culture of the LC isolate in heat-sterilized ethanol leaf extract of cocoyam.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.0000347 | 0.0000347 | 0.28 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 0.7905236 | 0.1581047 | 1282.34 | <.001 |
| Dilution_Ratio | 5 | 4.8789236 | 0.9757847 | 7914.31 | <.001 |
| Days.Dilution_Ratio | 25 | 0.4432347 | 0.0177294 | 143.80 | <.001 |
| Residual | 35 | 0.0043153 | 0.0001233 | | |
| Total | 71 | 6.1170319 | | | |

## APPENDIX 58

Variate: Mean dry weight of the culture of the LC isolate in heat-sterilized aqueous (water) leaf extract of plantain.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.0000500 | 0.0000500 | 0.11 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 1.0811611 | 0.2162322 | 462.88 | <.001 |
| Dilution_Ratio | 5 | 2.6273611 | 0.5254722 | 1124.86 | <.001 |
| Days.Dilution_Ratio | 25 | 0.1374056 | 0.0054962 | 11.77 | <.001 |
| Residual | 35 | 0.0163500 | 0.0004671 | | |
| Total | 71 | 3.8623278 | | | |

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## APPENDIX 59

Variate: Mean dry weight of culture of the LC isolate in heat-sterilized ethanol leaf extract of cassava.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.0011681 | 0.0011681 | 1.51 | 0.228 |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 0.7196916 | 0.1439383 | 185.49 | < 0.001 |
| Dilution_Ratio | 5 | 4.6972943 | 0.9394589 | 1210.65 | < 0.001 |
| Days.Dilution_Ratio | 25 | 0.4355027 | 0.0174201 | 22.45 | < 0.001 |
| Residual | 35 | 0.0271599 | 0.0007760 | | |
| Total | 71 | 5.8768319 | 0.0827723 | | |

## APPENDIX 60

Variate: Mean dry weight of culture of the LC isolate in heat-sterilized aqueous (water) leaf extract of cocoyam.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 0.007401 | 0.007401 | 5.78 | |
| Rep.*Units* stratum | | | | | |
| Days | 5 | 0.564774 | 0.112955 | 88.15 | <.001 |
| Dilution_Ratio | 5 | 4.013957 | 0.802791 | 626.50 | <.001 |
| Days.Dilution_Ratio | 25 | 0.207751 | 0.008310 | 6.49 | <.001 |
| Residual | 35 | 0.044849 | 0.001281 | | |
| Total | 71 | 4.838732 | | | |

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## APPENDIX 61

Variate: Mean number of sclerotia formed by the isolates after 14 days in heat-sterilized ethanol leaf extract of plantain.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 5.633.00 | 5.633.00 | 8.89 | |
| Rep.*Units* stratum | | | | | |
| Dilution_Ratio | 4 | 6.582.05 | 1.646.05 | 2.598.05 | <.001 |
| Isolates | 2 | 3.350.03 | 1.675.03 | 2645.05 | <.001 |
| Dilution_Ratio.Isolates | 8 | 7.132.03 | 8.915.02 | 1407.68 | <.001 |
| Residual | 14 | 8.867.00 | 6.333.01 | | |
| Total | 29 | 6.687.05 | | | |

## APPENDIX 62

Variate: Mean number of sclerotia formed by the isolates after 14 days in heat-sterilized ethanol leaf extract of cassava.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 4.033 | 4.033 | 0.81 | |
| Rep.*Units* stratum | | | | | |
| Dilution_Ratio | 4 | 798742.200 | 199685.550 | 40243.73 | <.001 |
| Isolates | 2 | 156.067 | 78.033 | 15.73 | <.001 |
| Dilution_Ratio.Isolates | 8 | 909.600 | 113.700 | 22.91 | <.001 |
| Residual | 14 | 69.467 | 4.962 | | |
| Total | 29 | 799881.367 | | | |

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## APPENDIX 63

Variate: Mean number of sclerotia formed by the isolates after 14 days in heat-sterilized ethanol leaf extract of cocoyam.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 1.633 | 1.633 | 1.05 | |
| Rep.*Units* stratum | | | | | |
| Dilution_Ratio | 4 | 936962.333 | 234240.583 | 1.500.05 | <.001 |
| Isolates | 2 | 1043.467 | 521.733 | 334.04 | <.001 |
| Dilution_Ratio.Isolates | 8 | 1180.867 | 147.608 | 94.51 | <.001 |
| Residual | 14 | 21.867 | 1.562 | | |
| Total | 29 | 939210.167 | | | |

## APPENDIX 64

Variate: Mean number of sclerotia formed by the isolates after 14 days in heat-sterilized aqueous (water) leaf extract of plantian.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 1.080.01 | 1.080.01 | 24.39 | |
| Rep.*Units* stratum | | | | | |
| Dilution_Ratio | 4 | 8.041.05 | 2.010.05 | 4.539.05 | <.001 |
| Isolates | 2 | 1.902.03 | 9.509.02 | 2147.27 | <.001 |
| Dilution_Ratio.Isolates | 8 | 9.435.02 | 1.179.02 | 266.30 | <.001 |
| Residual | 14 | 6.200.00 | 4.429.01 | | |
| Total | 29 | 8.069.05 | | | |

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**APPENDIX 65**

Variate: Mean number of sclerotia formed by the isolates after 14 days in heat-sterilized aqueous (water) leaf extract of cassava.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 425.6 | 425.6 | 0.89 | |
| Rep.*Units* stratum | | | | | |
| Dilution_Ratio | 4 | 889599.5 | 222399.9 | 466.61 | <.001 |
| Isolates | 2 | 2139.8 | 1069.9 | 2.24 | 0.143 |
| Dilution_Ratio.Isolates | 8 | 6430.9 | 803.9 | 1.69 | 0.187 |
| Residual | 14 | 6672.9 | 476.6 | | |
| Total | 29 | 905268.7 | | | |

**APPENDIX 66**

Variate: Mean number of sclerotia formed by the isolates after 14 days in heat-sterilized aqueous (water) leaf extract of cocoyam.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 1 | 1.633.00 | 1.633.00 | 0.68 | |
| Rep.*Units* stratum | | | | | |
| Dilution_Ratio | 4 | 1.007.06 | 2.519.05 | 1.041.05 | <.001 |
| Isolates | 2 | 5.363.02 | 2.681.02 | 110.84 | <.001 |
| Dilution_Ratio.Isolates | 8 | 1.941.03 | 2.426.02 | 100.28 | <.001 |
| Residual | 14 | 3.387.01 | 2.419.00 | | |
| Total | 29 | 1.010.06 | | | |