

CHAPTER ONE

INTRODUCTION

Sesamum indicum L. is an oil crop belonging to the family Pedaliaceae; it consists of 16 genera and 60 species. The most important producing countries are India, China, Sudan, Burma and Mexico (Bailey 1949). It is considered as the third crop in Sudan area-wide. It comes after Sorghum and penisetum. It is mainly grown in Sudan under rains only in sand in Western Sudan. It is also cultivated in wide clay soils in the central clay plain of Sudan, particularly in Gedarif and Damazin areas. It is also cultivated in small areas Nuba Mountains and Southern province. Sesame plays an important role in Sudan economy. It is exported in the form seeds, but Limited quantities are exported in the form of oil and seed cake. Sesame seeds are incorporated directly in human food by seed broadcasting on some kinds of bread and cake. It also mixed with sugar and flours in sweets industries. Sesame seed contains 50% of its weight as oil with yellow color resistant to rancidity. The pure oil is used in cooking and fat industry. Lower quality oil is used in soap industry. Sesame seeds contain 20- 25% digestible protein by 92%.

Tap root of the crop is deep and reaches the depth 90 Cm. stem is erect with several branches and average length 80-110 Cm, covered with white hairs, angular and solid in the vegetable stage and becomes yellow at maturity. The leaf is oval or lanceolate, with dentate margin. Leaves are carried on long petioles 5 Cm and have no stipules (exstipulate).The suitable environmental condition of the crop is humid weather (rains 300-800 mm) during the growing season.

The suitable temperature is 25-27°C. The higher productivity was recorded in Gedarif area in a successful rainy season (500- 600mm).

The crop could be produced in different types of soil but the most suitable is light soil with good drainage (Khatab et al., 2001) Arabic text.

The crop is attacked by many diseases world, the most important seed - borne disease. Leppuk and Sowell (1964) reported that *Alternaria sesami* is a common seed- borne pathogen of sesame samples collected throughout the world. The pathogen was distributed by shipping seeds from one country to another. Nobel Richardson (1968) reported that *Alternaria sesami*, *Cercospora sesami*, *Macrophomina phaseolina*, *Drechslera sesami*, *Cylindrosporium sesami*, *Corynespora cassiicola*, *Fusarium oxysporum* *Fusarium Spp* and *Phytophthora nicotianae* are considered as seed- borne fungi of sesame (*Sesamum indicum L.*). Daftari and Verna (1973) reported the seed-borne infection of sesame seed with *Fusarium solani*. Mathur and Kabeere (1975) reported that *A. sesami*, *Fusarium moniliform* and *C. cassiicola* newly recorded for Uganda together with *Gibberella*, *Fujikuroi* *Mycosphaerella sesami*, *Fusarium oxysporum* and *verticillium dahlias* were isolated from four Ugandan seed samples. In Sudan, Salih (1985) has shown that 13 seed-borne fungi were recounted in 25 seed samples of sesame *Alternaria sesame*, *A. alternata*, *A. longissima*, *Macrophomina phaseolina*, *Cercospora sesami*, *Fusarium oxysporum*, *F. moniliform*, *Pithomyces sacchari*, *Chaetomium spinosum*, *Drechslera spicifer*, *D. rostrata* and *Curvularia lunata*. In Sudan also Kamees and Schlosser (1990) have shown that the testing of 165 Sudanese sesame seeds showed that although seed-borne pathogen such as *Macrophomina phaseolina* and *Alternaria sesamicola* were wide spread in the country their incidence was generally at low level, while other fungi which infect crops but not sesame were *Phome sorghina*, *Ascochyta gossypii* and *Fusarium moniliform* were often found on sesame seed also showed the saprophytic

mycoflora which include 41 fungal species, *Asperillus flavus* *A. ngier* were present in 77% of samples. The study was designed to detect incidence of seed –borne fungi in general, seed –borne pathogen of sesame and study the probability of introduction of seed –borne diseases with imported seed (*Drechslera rostrata*, *Fusarium oxysporum*, *Alternaria sesami*, *Asperigllus ngier*, *A. flavus* and *Penicillium digitatum*).

Objective of this study:-

1. To determine seed- borne pathogens in the seed samples of the three tested cultivars.
2. To determine the pathogenic propensities of the most prevalent pathogens isolated from seeds.
3. To control fungi seed-borne on the seeds of the three cultivars tested by biological and chemical methods.

CHAPTER TWO

LITERATURE REVIEW

2.1 Sesame crop:

Sesame (*Sesamum indicum* L.) is one of the most important oilseed crops worldwide, and has been cultivated in Korea since ancient times for use as a traditional health food. Sesame seeds are used in the making of tahin (sesame butter) and for the preparation of rolls, crackers, cakes and pastry products in commercial bakeries. There are numerous varieties and ecotypes of sesame adapted to various ecological conditions. However, the cultivation of modern varieties is limited due to insufficient genetic information. Many farmers continue to grow local sesame (Souza *et al.*, 1991). Sesame plays an important role in human nutrition. Most of the sesame seeds are used for oil extraction and the rest are used for edible purposes (El Khier et al, 2008). Sesame is grown primarily for its oil-rich seeds. Before seeds were appreciated for their ability to add nutty flavour or garnish foods, they were primarily used for oil and wine (Gandi, 2009). Sesame seed is harvested when about 50% of capsules turn yellow in colour from green. Other indications of the optimum time for harvesting (physiological ripeness) include; lowest capsules turning brown and beginning to pop open, stem turning yellow, leaves beginning to fall off, end of blossoming, leaves turning yellow (Kimbonguila et al., 2009).

2.1.1 Classification:

Kingdom: Plantae
Unranked: Angiosperms
Unranked: Eudicots
Unranked: Asterids
Order: Lamiales
Family: Pedaliaceae
Genus: *Sesamum*
Species: *Indicum*
S.N: *Sesamum indicum* (Bailey 1949).
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2.1.2 Botanical:

Erect m, stout , aromatic ,annual herb up to 2m tall, root system with strongly tapering taproot up to 90cm long , bearing many laterals , stem firm , square with ribs at each corner , up to 3 cm in diameter at base , bright pale green , sparsely hairy to glabrous , with 4-celled glands present on all parts. Leaves decussately opposite in lower parts , arranged spirally and 3-lobed to 4-folioiate in upper parts stipules absent, petiole up to 17 cm long grooved above, at least at base, blade of lowest leaves ovate in outline 10-12cm x 5-13cm, margin entire or partly toothed .Flowers in small fascicles in upper leaf axils bisexual , zygomorphic , 5-merous , with 2 bracts at base , each bract with an auxiliary gland , calyx with oblong lobes 4-7mm x 1-1.5 mm , slightly fused at base , apex acute long – hairy , corolla campanulate , 2-3.5 cm long , base slightly bent and widened, slightly 5-lobed, with lobes. Fruit an oblong quadrangular capsule 1.5-3 cm long hairy, with a short triangular beak at apex, grey –

brown at maturity, loculicidally dehiscent, many seeded. Seeds flattened bovid, 2-3mm long , 0.5-1 mm thick , narrowly ridged all round, rather smooth , white, ivory grey beige, brown red or black. Seedling with epigeal germination (Singh1952).

2.1.3 Origin and Distribution:

Discussion continues about the exact origin of sesame. It is often asserted that sesame has its origin in Africa and spread early though west Asia, China and Japan, which themselves became secondary centers of diversity. With the exception of *Sesamum prostratum* Retz, all the wild *Sesamum* species are found in Africa (Purse glove 1977).this variability and the importance of sesame in the economies of several African countries could further justify the African continent to be ultimate centre of origin. Howere, Bedigian (2004) demonstrated that the crop was first domesticated in India, citing morphological and cytogenetic affinities between domesticated sesame and the south India native *S. mulayanum* Nair, as well as archeological evidence that it was cultivated at Harappa in the Indus valley between 2250 and 1750 BC. All these assertion make it difficult to say with certainty the exact origin of the crop. Due to its relatively low productivity sesame ranks only ninth among the top thirteen oilseed crop, which up 90% of the world production of edible oil.

2.1.4 Grown in Sudan:

Sixty percent of the production of in Sudan is grown rain fed mechanized like Gedarif, Cassata, Blue Nile, Sinner, White Nile and North Kordofanian. Traditionally about 40% the areas are Blue Nile Sinnar White Nile Kodofan, North, West and South Darfor North, west and Southern regions (Bank of Sudan 1981-2003).

2.1.5 Economic Important:

In the Sudan, sesame is very important both for local consumption and export. Locally, oil is extracted by expeller or by traditional wooden mills driven by camels. The oil is used for cooking purposes. A small percentage of oil is also used in pharmaceuticals, cosmetics and perfumery industries and for manufacture of soaps, paints and insecticides. The oil can be readily hydrogenated for use in margarines, shortening and vanaspati (Patterson 1983). Sesame seed and kernels are also used for the preparation of sweets, as condiment for culinary purpose and for confectionery and bakery products. Seed are also consumed as reacted sesame seed paste (Tahina). Sesame cake is rich in cattle, the de hulled and de fatted meal is usually fed to as portion supplement because of the high yield and quality of the oil and meal, sesame is often called the queen of the oil seed (weiss1971). The importance of sesame as source of edible oil and high quality protein is continuously increasing. Sesame plays an important role in human nutrition. Most of the sesame seeds used for oil extraction and the rest are used for edible purposes (Elleuch et al, 2000).

2.2 Seed-borne fungi of sesame:

Leppuk and Sowell (1964) reported that *Alternaria sesami* is a common seed-borne pathogen of sesame samples collected throughout the world. The pathogen was distributed by shipping seeds from one country to another. Nobel Richardson (1968) reported that *Alternaria sesami*, *Cercospora sesami*, *Macrophomina phaseolina*, *Drechslera sesami*, *Cylindrosporium sesami*, *Corynespora cassiicola*, *Fusarium oxysporum*, *Fusarium Spp* and *Phytophthora nicotianae* are considered as seed-borne fungi of sesame (*Sesamum indicum L.*) Daftari and Verna (1973) reported the seed-borne infection of sesame seed with *Fusarium solani*. Mathur and Kabeere (1975) reported that *A. sesami*, *Fusarium moniliform* and *C. cassiicola* newly recorded for Uganda together with *Gibberella*, *Fujikuroi*, *Mycosphaerella sesami*, *Fusarium oxysporum* and *verticillium dahliae* were isolated from four Ugandan seed samples. Kushi and Khare (1979) reported that *Fusarium equiseti*, *Phoma Spp* and *Cephalosporium Spp.* were encountered in sesame seed in India. Also the same author has shown that among (26) a sample of sesame seed *Macrophomina phaseolina* was associated with 23 samples, *Corynespora cassiicola* with 11 and *Alternaria sesami* with 10 and the three isolates were pathogenic resulting in seed rot and post-emergence losses, stem rot and leaf spots. Yu et al (1982) have shown that *A. sesami*, *A. alternata* and *A. longissima* were recorded as seed-borne in Korean seed samples.

Rajagopalan and Shanmugam (1983) isolated *A.carthmi* from surface disinfected sesame seed samples in India. It has been reported that *Drechslera neergardii* and *Phoma lobulosa* were recorded in sesame seeds in India (Reddy and Reddy, 1982). Singh and Singh (1983) reported that 108 of seed samples from crop grown in 1974-77, 65 contained seeds with Microsclerotia of *Macrophomina phaseolina* and

incubation tests yielded 24 fungal spp. Most were saprophytes but some important pathogens including *A. sesami*, *Cephalosporium acremonium*, *Fusarium oxysporum f. sp. sesami* and *F. solani* and presence of *Phytophthora nicotianae* var. *parasitica* was detected in microtome section of seed. Wu (1988) has shown that Blotter method detected ten fungi on sesame seed samples in Taiwan five of these *A. longissima*, *A. sesami*, *A. alternata* and *Corynespora cassiicola* are newly reported on sesame in Taiwan. Recently in Iran Ershad Riahi (2000) reported that thirty four fungal species of 15 genera *Acremonium*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Rhizopus*, *Trichoderma* and *Tiarosporella* were identified from seed of 17 sesame cultivars in an experiment conducted in Iran.

In Sudan, Salih (1985) has shown that 13 seed-borne fungi were recounted in 25 seed samples of sesame *Alternaria sesame*, *A. alternata*, *A. longissima*, *Macrophomina phaseolina*, *Cercospora sesami*, *Fusarium oxysporum*, *F. moniliform*, *Pithomyces sacchari*, *Chaetomium spinosum*, *Drechslera spicifer*, *D. rostrata* and *Curvularia lunata*. In Sudan also Kamees and Schlosser (1990) have shown that the testing of 165 Sudanese sesame seeds showed seed-borne pathogen such as *Macrophomina phaseolina* and *Alternaria sesamicola* were wide spread in the country. Their incidence was generally at low level, while other fungi which infect crops but not sesame were *Phoma sorghina*, *Ascochyta gossypii* and *Fusarium moniliform* were often found on sesame seed also showed the saprophytic mycoflora which include 41 fungal species, *Asperillus flavus*, *A. niger* were present in 77% of samples. Tarr (1955) recorded a numbers of fungal diseases in Sudanese sesame crop but did not show whether these diseases were transmitted by seeds or not. These are leaf spot caused by *Cercospora sesame* and powdery mildew which

was found to be caused by *Levellula taurica*, *Spharotheca fuliginous* and *Oidium Spp.*

Yu et al., (1982) reported that brown to black lesions on the leaves and capsules were produced on the infected sesame plant. Leppik and Sowell (1964) *Alternaria sesami* has been known as causal of sesame blight in the United States. Also Berry (1960) reported that this fungus causes poor generation, post- emergence damping –off, leaf and stem spots, premature defoliation and death of plant. Mathur and Kabeere (1975) showed that seed - borne infection by *A. sesami* caused heavy seed rot and seedling death. More recently, Yu et al, (1982) demonstrated that *A. sesami* produced severe foliage and stem infection on one- month old sesame plant.

Malguti and Cicerone (1967) showed that *A. sesami* produced concentric irregular leaf spots. Culp and Thomas (1964) reported that *A. sesami* is carried on in the seed and thus, badly reduced germination. According to Leppik and Sowell (1964) *A. sesami* from infected pods penetrates into seed coat and remains viable until germination of the seed. As its spores attach to the seed and contaminate the Warpping material, the pathogen could be carried and disseminated rather efficiently. Moreover, Leppik and Sowell (1964) have shown that germination tests of numerous seed samples collected throughout the world proved that *A. sesami* is a common seed-borne pathogen distributed frequently by shipping seeds from one country to another. Singh et al., (1974a) recorded that out of a total of almost 90% infection of *A. sesame* in *Sesamum indicum*, only 13% were embryo infection. The remaining 87% were found to be mostly seed coat infection. A few records have been reported about the pathogenic nature of *A. sesame*. Mohanty and Behera (1958) have shown that *A. sesame* isolated from infected leaves is pathogenic when

inoculated again in its host. Berry (1960) reported that *A. sesame* isolated from sesame plants was non-pathogenic to other crop plants. Deshpande and Shinde (1976) studied two isolate of *A. sesame* from diseased sesame plant and proved that one of them of was more virulent than the other.

2.3 Bacterial Diseases:

Bacterial leaf spot resulting from infection by *Xanthomonas sesami* produces light- brown, darkish, angular spots with purple margin. The spots are generally located between the leaf veins, but can advance along the vines and petioles, when they become dark -brown to purple shiny lesions. The spots often coalesce to form large necrotic areas on the leaves. Spots on pods are usually slightly sunken, purplish and shiny. It is known in the Sudan as *Marad ed Dum*, blood disease due to the red colour of the infected plant tissue (Surinam 1964).

2.4 Nematodes:

Sesame is generally believed to be highly resistant or immune to *Melodogyne spp* root- knot nematodes and also perhaps to other species in the U.S.A. In Iran, however, *M. marioni*, locally known as nematode risheh also attacks tobacco and other local crops included in rotation with sesame and damage in specific years could be severe. There is some indication from South American countries that infection by *Rhizotonia Spp.* and *Fusarium spp.* can be associated with high nematode populations, but this association has not been proven. Mature plants may be attacked by termites, more often in dry regions, in seasons of below-average rainfall, or in clean-weeded crops. Many small rodents, small buck and other animals have a liking for sesame seed, and breeding or seed-production plots may require fencing against their

depredations. Birds can be particularly harmful to irrigated sowing grown out of the normal season in the arid tropics. (Hussain1970).

2.5 Identification of Drechslera Species on seeds:

Habit characters of 26 species of *Drechslera* observed on seed of different tropical and temperate crops in the standard blotter method and their colony characters on agar have been described with illustrations. The diagnostic feature of species is discussed. It is hoped that this pictorial of species guide will help greatly in their identification during seed health testing. The species studied were *Drechslera avene*, *D. oryzae*, *D. rostrata*, *D. gramineae*, *D. maydis*, *D. saccharii* (Chidambaram1969- 1971).

2.6 Botanical controls:

The antifungal effect of certain medicinal and aromatic plants extracts have been investigated by many workers (Singh and Dwivedi, 1987); Handique and Singh (1990). Thus, the development of new and different antimicrobial agents more safe has been a very important step (Agrafotis, 2002). However, the step of validation of traditional uses of antimicrobial compounds in higher plants was studied by a number of researchers. Accordingly, the effect of different plants extracts on the germination and growth of many fungal pathogens have been reported (Agrafotis, 2002).

The use of plant extracts for controlling *Fusarium* Spp, cultural practices and the use of other methods are the most common strategies. However, they are either not available or effective. The uses of natural products for the control of fungal diseases in plant are considered as an interesting alternative to synthetic fungicides due to their less negative impacts on the environment. Chand and Singh(2005).reported that the plant extracts,

VIZ *Calotropis procera*, *Eucalyptus globulens*, *Jatropha multifida*, *Azadirachta indica*, *Allium sativum* were significantly pronounced in reducing wilt incidence in *Cicer arietinum* L. Mycelial growth of various *Fusarium* species were inhibited by the plant extracts of *Adhatoda vasica*, *Azadirachta indica*, *Cinnamomum camphora*, and *Ocimum sanctum* (Prasad and Ojha, 1986); *Agave Americana*, *Cassia nadosa* Redd and Reddy, (1987); *Azadirachta indica* (Eswaramoorthy et al., 1989); *Azadirachta indica*, *Atropa belladonna*, *Calotropis procera*, *Eucalyptus amgdalline*, *Ailanthus excelsa* and *Lantana camara* (Bansal and Rajesh, 2000). Nwachukwu and Umechuruba (2001).

Also Singh and Hair Chand, (2004) reported that Leaf extract of *Azadirachta indica* at 100/con completely inhibited germination of pathogen spores.

2.7 Neem Tree:

Neem is versatile tree, it is considered to be one of the most promising trees of the 21 century. It has great potential in the field of pest management, environment protection and medicine. Also it has showing great promise as potential fertilizer (Abdalla, 2010).

2.7.1 Taxonomy:

Kingdom: Plantae

Division: Magnoliophyta

Order: Rutales

Suborder: Rutineae

Family: Meliaceae

Genus: *Azadirachta*

Species: *Azadirachta indica*

S.N: *Azadirachta indica* A.Juss

E.N: Neem

A.N: نيم

(Vietmeyer, 1992, and Schmutterer, 2002)



Plate .1:Neem leaves:

2.7.2Chemistry of Neem tree:

All parts of *Azadirachta indica* tree have been examined by chemists which contains number of chemical compound culled "triterpeness" or limonoids .there are nearly 100 proto limonoids, limonoids or triterpenoid, pentanor, hexane or triterpenoid and some none terpenioid Limonoids occurring in neem are related to nine different basic structure groups such as the azadiron, amoorastanin, vepinin and vilasinin, and seco sestems related to gedunin, Nimbin, nimbidin and salanin and the azadirachtin group which in fact belong lasically to the nimbolinin (Schumtterer,1995).the neem oil contains several terpenioid, steroids, alkaloids, flavonoids, glycoside and others (Anonymous,2001). The most important bioactive principle is Azadirachtin (Schmutterer, 1990). Azadirachtin is naturally found in neem kernel depending on the method of extraction (Anonymous, 1999b).

2.7.3 Distributions:

Neem is widely distributed throughout South East Asia and West Africa and part of Central America (Stoll, 2000). Neem is introduced to Sudan in the 20 century. The first one were planted at Shambat in 1916, today trees are spread in town and villages along the Blue and White Nile, irrigated areas of Central Sudan, Kordofan and Darfur (Schmutterer, 1969).

2.7.4 Active Ingredients:

The Neem tree produce a compounds of many active ingredients called Azadirachtin and it is tetramer titer penoids compound which influences the hormonam, feeding activity reproduction and fling ability of insect. Azadirachtin hl systeas low mammalian toxicity. It degrades rapidly in the environment and has low side effects on non-target species and beneficial insects. Seeds of the Neem tree contain the highest concentration of Azadirachtin. Salanin inhabits the feeding of wider any of insect pests, Nimbin and Nimbidin showed antiviral effects (Ganguli, 2002).

2.7.5 Mode of action:

Neem acts as insects feeding deterrent and growth regulator, the treated insects usually cannot molt to its next life stage and dies, Azadirachtin is chemically similar to ecdysone responsible for triggering molts. It also acts as repellent when applied to plant and does not produce a quick knock down and kill (Schmutterer, 1990).). Also Neem has some systemic activity in plants, its most effectively growing immature stages and adults are not killed by the growth regulator properties of Azadirachtin, but mating and sexual communication may be disrupted which results in reduced fecundity (Schmutterer, 1990 and Pedigo, 1999).

2.7.6 Uses of Neem in pest and disease control:

Neem is deemed very effective in the treatment of scabies although only preliminary scientific proof exists which still has to be corroborated and is recommended for those who are sensitive to Permethrin. A known insecticide which might be irritants and also the scabies mite has yet to become resistant to Neem, so in persistent cases Neem has been shown to be very effective, there is also anecdotal evidence of its effectiveness. In treating infestations of head lice in humans, it is also very good for treating worms (soak the branches and leaves in lukewarm water and drink it). In the traditional medicine Neem trees originated on the Indian subcontinent. The Neem twig is nature's tooth brush to over 500 million people daily in India alone. Herbal medicine is the oldest form of therapy practiced to be mankind and much of the oldest medicinal use of plants seems to have been based on highly developed 'dowsing instinct' (Grigs, 1981).

2.8 Damas:

Family Combretaceae comprises about 20 genera and about 600 species found in tropical and subtropical regions of the world. The family has few genera with great economic value, a useful timber is obtained from some species belong to it and other species has medicinal importance. Damas *Conocarpus lancifolius*Engle is one of the most important species in this family (Pandey and Misra, 2008).

2.8.1 Classification:

Kingdom: Plantae

Phylum: Tracheophyta

Class : Magnoliopida

Order : Myrtales

Family : Combretaceae

S. N. : *Conocarpus lancifolius* Engl.



Plate.2: *Conocarpus lancifolius* plant.

2.8.2 Distribution:

Natural stand of Damas are found beside intermittent watercourses of northern Somalia and in the southwest part of the Arabian Peninsula. Some of these streams are salty and some sulphurous. The tree is also cultivated in Somalia, as it is in Djibouti, Sudan, Kenya, north and south Yamane, and Pakistan. A small plantation has been established in Sudan Khashm El-girba arboretum. About 10000 trees have been planted successfully in limestone near Mombasa, Kenya (Grigs, 1981).

2.8.3 Economic importance:

Conocarpus lancifolius is multipurpose; wood which is the main product is used domestically for house construction, firewood and excellent charcoal. Commercially timber was more useful formerly; it was cut and exported from Somalia to Arabia for dhow construction. Other potential uses include wood based board. Bark may be a useful source of tannins (Booth and W, 1993).

Information on the importance of *C. lancifolius* in its native distribution areas relative to other species with similar wood, fuel and forage uses is lacking hence it is difficult to assess its importance. However Somali tribes owing the Damas (Tugs) dry river valleys (wadés) containing *C. lancifolius* have restricted cutting because of the threat of overexploitation (Booth and Wickens, 1993).

2.9 Chemical control:

Tilt: it's one fungicides use control of fungi of case crops in field.

2.9.1 Mode of action:

The mode of action of Tilt is as a demethylation inhibitor of sterol biosynthesis (DMI) which disrupts membrane synthesis by blocking demethylation. Fungal pathogens can develop resistance to products with the same mode of action when used repeatedly (Erwin1973).

2.9.2 Uses of Tilt:

Control of leaf diseases protecting the flag leaf is important for Rust maximizing the potential yield. Highest (*Puccinia* spp.) yields are normally obtained when Tilt Powdery Mildew is applied when the flag leaf is 50% to (*Blumeria* spp., fully emerged *Erysiphe* spp) . Mummyberry Disease 6 Make first application of Tilt beginning at (*Monilinia* green tip and repeat in 7-10 days. Tahani (2005) reported that seed –borne fungi control aqueous Drechslera Spp. Reported that seed –borne fungi caused 20-30 per cent reduction in germination in sunflower (Vir1983).

CHAPTER THREE

Material and methods

This study was conducted under laboratory conditions at Plant pathology Department, College of Agricultural Studies “Shambat”, Sudan University of Science and Technology (SUST) within the period September to December 2013, for seed health tested of three cultivars of *Sesamum indicum L.* in three localities in the Sudan (Gadarif, Jazera and Radoom Beladi) and antifungal activity of Neem and Damas leaves aqueous extracts and efficacy of fungicide, Tilt 250 EC, against *Drechslera rostrata*.

3. The materials and equipment used in this study are listed below:

3.1 Equipments:

Seeds of Sesame

Paper bags

Bandage

Needle laminar

Petri dishes (9cm) Autoclave

Conical flasks Incubator

Sensitive balance Centrifuge

Microscope

Gloves Camera

Marker pen Medical cotton

3.2 Materials:

Potato dextrose agar

Tilt fungicide 250EC

Infected plant

Neem leaves

Damas Leaves

Distilled water

Sodium hydrochloride (3%)

3.3 Collection of samples:

Samples of sesame (*Sesamum indicum* L.), were collected from different parts of the country from the crop of the season (2013-2014) the samples were collected namely from (Gadarif, Jazera and Radoom). The samples were collected in paper bags and transferred to the lab for investigations.

3.4 Detection and isolation of the seed-borne fungi:

3.4.1 The Dry Seed Inspection:

Two hundred of seed samples from each cultivar were inspected by naked eyes or hand lens. Seed were divided to two categories:-

A: Healthy: were pure seed.

B: Unhealthy: were weed seed, broken, wrinkled, and discoloured.

3.4.2 The Blotter Method:

Sterilized four moistened blotter papers were placed in sterilized glass Petri-dishes. Seeds were plated 25 seed per plate under sterilized condition. Plates are then incubated at room temperature 25-28^{0c} for 7days. Plates examined by steriobinocular microscope and compound microscope on 8th day. In this method there is pretreatment for the seed.

3.4.3 The Agar Method:

In this method we use potato dextrose agar (PDA) medium. The medium is sterilized on autoclave for 15minutes under 15 atmosphere pressures under 121°C. The medium is poured in the sterilized glass Petri-dishes when the medium temperature is 45°C. The plate is then used under sterilized condition in the laboratory for plating the tested seeds. Twenty five seed/glass Petri-dishes were incubated 7days under alternating cycles of light and darkness **and** room temperature in range 20-25°C .On the day 8th the glass Petri-dishes are examined by compound microscope. The fungi detected were identified at the Faculty of Agricultural Studies “Shambat”, Sudan University of Science and Technology. The plated seed were pretreated by sodium hypochlorite 3% to exclude saprophytes.

3.5 Pathogenicity:

The fungi *Drechslera rostrata*, *Fusarium oxysporum* and *Asperigillus niger* were grown on sterilized barley medium. After 10 of inoculation on barley, one tea spoonful was transferred to the sterilized soil. Water was added to absorb fungi at 5days. Forty seeds were taken from three categories of the sesame samples. Three replicates were made. The pots irrigated and left for ten days for pathogen establishment, percentages of

diseases incidence pre- emergence and post- emergence and then the results were recorded

3.6 The Chemical control:

3.6.1 Effect of fungicides on linear growth of *Drechslera rostrata* in vitro:

Chemical tested was Tilt. The different concentrations were (5ppm, 10ppm 15ppm). Firstly, the concentration 5% of Tilt was taken and added to the sterile Distilled water to give 100ml, and secondly, the concentration 10% of Tilt was taken and added to the sterile Distilled water to give 100ml, thirdly the concentration 15% of Tilt was taken and added to the sterile Distilled water to give 100ml. Ten ml was taken by injection from each concentration and added to the medium. Each concentration was replicated three times (to give 9 medium). Sample of fungus (*Drechslera rostrata*) was taken by lap and cultivated at the central of the glass Petri-dishes medium and the Petri-dishes were then incubated at 30 °C and the growth of the fungus was calculated after every 2days and taken the record by Centimeter

3.7 Biological control:

3.7.1 Effect of plant Extracts on linear growth of *Drechslera rostrata* vitro:

Two types of leaves tree from (Neem, Damas) were collected and dried. Five grams from the two powders were taken and added distilled water to give 100ml. Ten grams were taken from each powder and added to the distilled water to give 100ml. Fiveten grams were taken from each powder

and added to the distilled water to give 100ml and finally were left it over twenty four hours and filtrated by bandage. Each concentration were taken 10ml by injection and added to 90ml medium in flask. And distributed in six sterile Petri-dishes of concentration 5% three for Damas and three for Neem, concentration 10% taken six Petri-dishes three for Damas and three for Neem and concentration 15% taken six Petri- dishes three for Damas and three for Neem. Taken sample of fungus (*Drechslera rostrata*) by wereloop and cultivated at the central of the glass Petri-dishes medium and the Petri- dishes were then incubated at 30°C and the growth of the fungus was after every 2days and taken record by centimeter.

The effect of each extracts was calculated as percentage of reduction in diameter of fungal growth (R) where: -

$$R = \frac{dc - dt}{Dc} \times 100$$

Dc

Where R = Percentage reduction of the growth, dc= diameter of controlled growth and dt= diameter of treated growth.

Sometime it Inhibition Zone

3.8 Experimental design:

These experiments were arranged in a Complete Randomized Design

3.9 Statistical analyses:

The obtained data was statistically analyzed according to analysis of variance (ANOVA); -Duncan's Multiple Range Test was used for mean separation.

CHAPTER FOUR

RESULTS

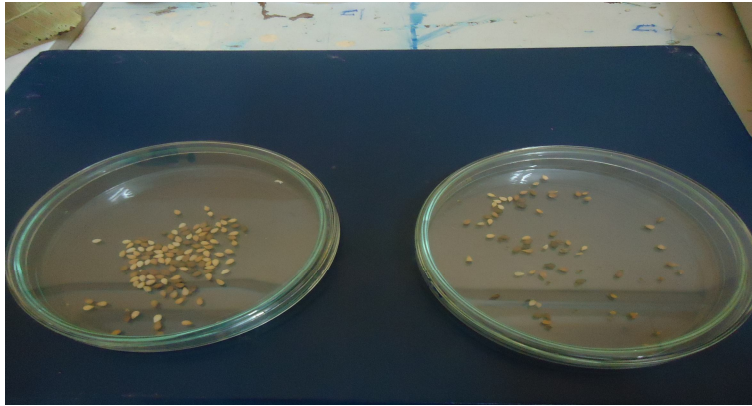
4-1 Detection of seed –borne fungi in sesame:

4.1.1 Dry inspection:

Three cultivars of sesame of 2012- 2013 were examined namely (Gadarif, Jazera and Radoom Beladi). The result revealed the percentage of pure healthy and unhealthy, broken, weeds, discoloured and malformed seeds. Gardarif was best percentage of healthy is 71%, 70% in Radoom Beladi and 65% in Jazera and unhealthy percentage 29% in Gardarif, 29.5% in Radoom Beladi and 35%in Jazera. As show in table (1) and Plate (1)

Table (1) Incidence of different categories of the cultivars tested (Dry inspection test) 200 seeds tested:

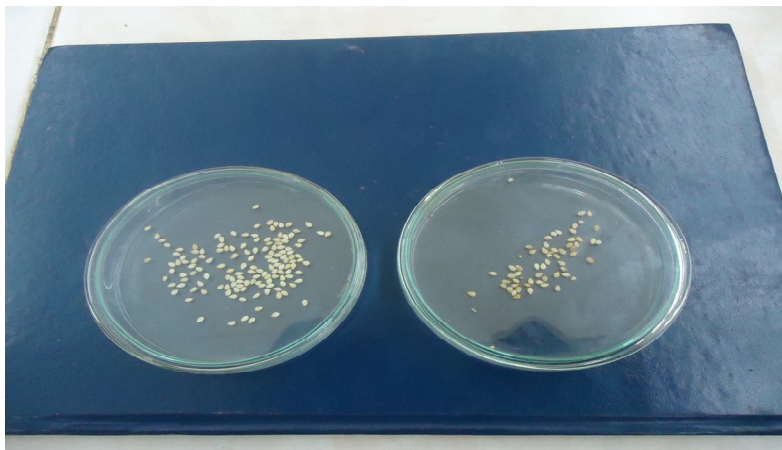
<i>Sample</i>	<i>Healthy</i>	<i>Unhealthy</i>
<i>Gadarif</i>	71%	29%
<i>Jazera</i>	65%	35%
<i>Radoom Beladi</i>	70.5%	29.5%



Healthy seeds

Jazera

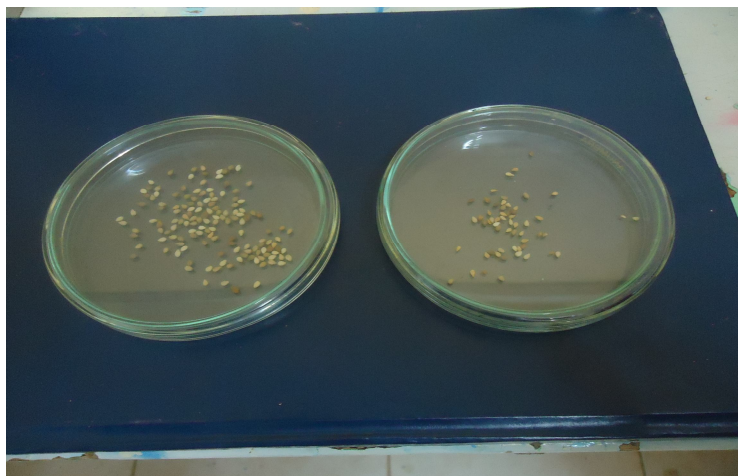
Unhealthy seeds



Healthy seeds

Gadarif

Unhealthy seeds



Healthy seeds

Radoom Beladi

Unhealthy seeds

Plate (3) Dry inspection of the three seed sample of Sesame:

4.1.2 The Blotter test:

Three sesame seed samples cultivars of (Gadalf Jazera and Radoom Baled) in using blotter method in case of sesame seed, the following fungi were detected: (*Drechslera rostrata*, *Fusarium oxysporum*, *Alternaria sesami*, *Asperigillus ngier*, *A. flavus* and *Penicillium digitatum*). The percentage of disease incidence *Derchsletra rostrata* 21%, 17.5% and 17.5%, *Fusarium oxysporum* was detected in the range 12.5%, 14.5% and 15%, *Alternaria sesami* was in the range 11%, 12% and 10%, *Asperigillus ngier* and *Asperigillus flavus* were detected in the range 6.5%, 11.5%, 6.5%, 8%, 6.5% and 8%and *Penicillium digitatum* was detected in the range 10%, 6%and 10.As shown in table (2) and plate (2),(3).

Table (2) Diseases incidence of fungi detected in the three sample of sesame by standard Blotter method 200seeds were tested for each seed sample (non pretreated) in 100%:

Cultivars	Fungi					
	D.r	F.o	Al.s	P.d	A.n	A.v
Gadarif	21%	12.5%	11%	10%	6.5%	6.5%
Jazera	17.5%	14.5%	12%	10%	8%	6.5%
Radoom Beladi	17.5%	15%	10%	6%	11.5%	8%

D.r: *Drechslera rostrata*

F.o: *Fusarium oxysporum*

Al.s: *Alternaria sesami*

P.d: *Penicillium digitatum*

A.n: *Asperigllus ngier*

A.v: *Asperigllus flavus*

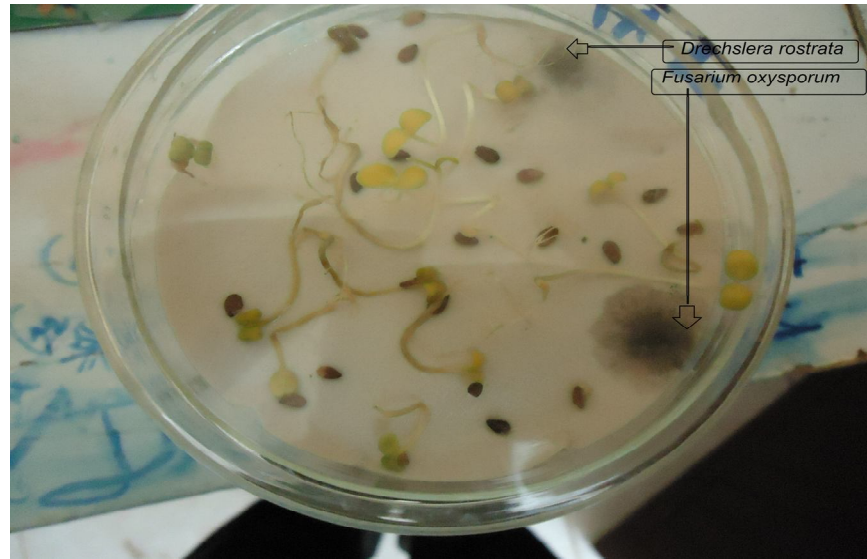


Plate (4) fungi detected on blotter:

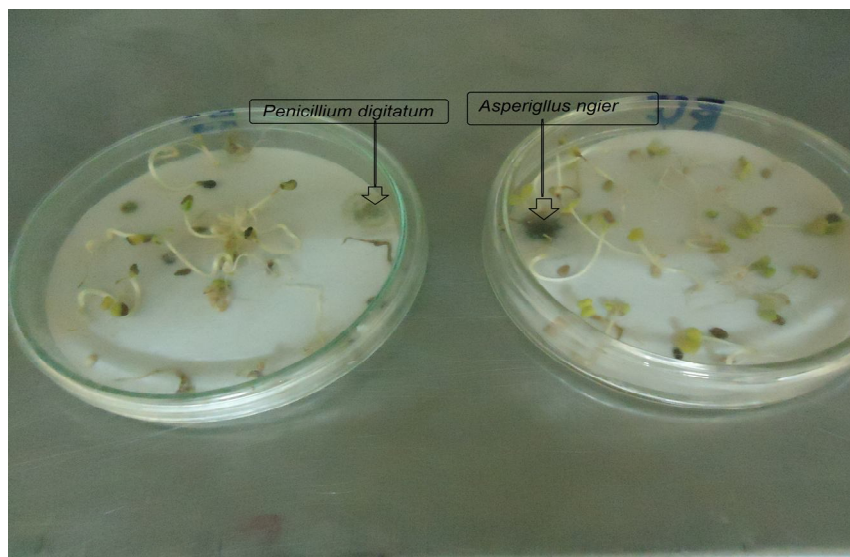


Plate (5) fungi detected on blotter:

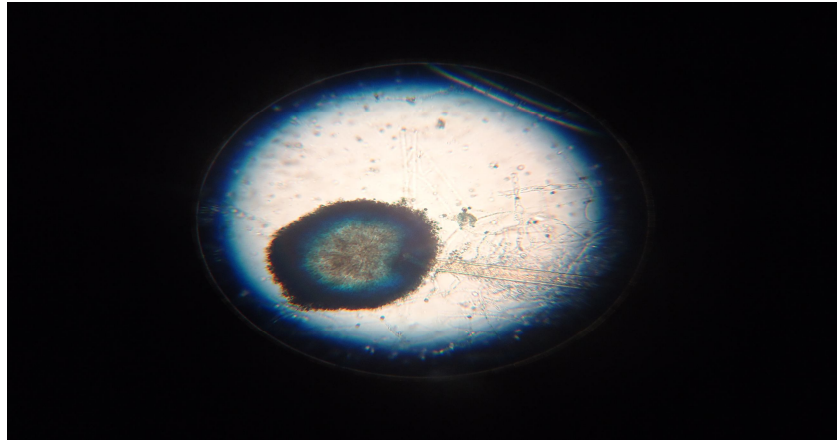


Plate (6) *Asperigillus ngier* Magnification (x40):

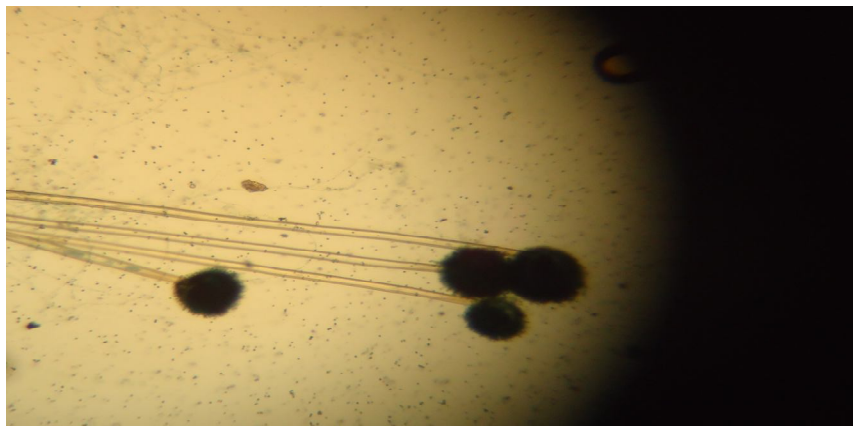


Plate (7) *Asperigillus flavus* Magnification (x40):

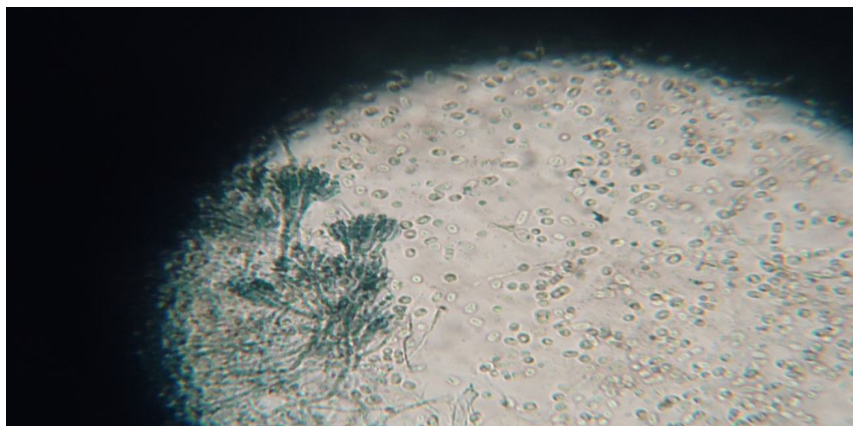


Plate (8) *Penicillium digitatum* Magnification (x40):

4.1.3 The Agar test:

Three sesame seed cultivars (Gadarif Jazera and Radoom Baled) using in Agar method in case of sesame seeds, the following fungi detected: (*Drechslera rostrata*, *Fusarium oxysporum*, *Alternaria sesami*, *Asperigillus ngier* *A. flavus* and *Penicillium digitatum*). *Drechslera rostrata* was detected in high percentage 22%, 18.5% and 19%, *Fusarium oxysporum* was detected in the range 5%, 15% and 15%, *Alternaria sesami* was in the range 14%, 9% and 13%, *Asperigillus ngier* and *Asperigillus flavus* were detected in the range 10%, 10% .6.5% .8.5%,6.5% and 10% and *Penicillium digitatum* was detected in the range 7.5% and 7.5% . As shown in table (3) and plate (4),(5) ,(6)

Table (3) Diseases incidence of fungi detected in the three sample of sesame by standard. PDA method 200 seeds were tested for each seed sample (non-pretreated):

Cultivars	Fungi					
	D.r	F.o	Al.s	P.d	A.n	A.v
Gadarif	22.5%	17.5%	14%	-	10%	10%
Jazera	18.5%	15%	9%	7.5%	10%	6.5%
RodoomBeladi	19%	15%	13%	7.5%	8.5%	6.5%

D.r: *Drechslera rostrata*

F.o: *Fusarium oxysporum*

Al.s: *Alternaria sesami*

P.d: *Penicillium digitatum*

A.n: *Asperigllus ngier*

A.v: *Asperigllus flavu*

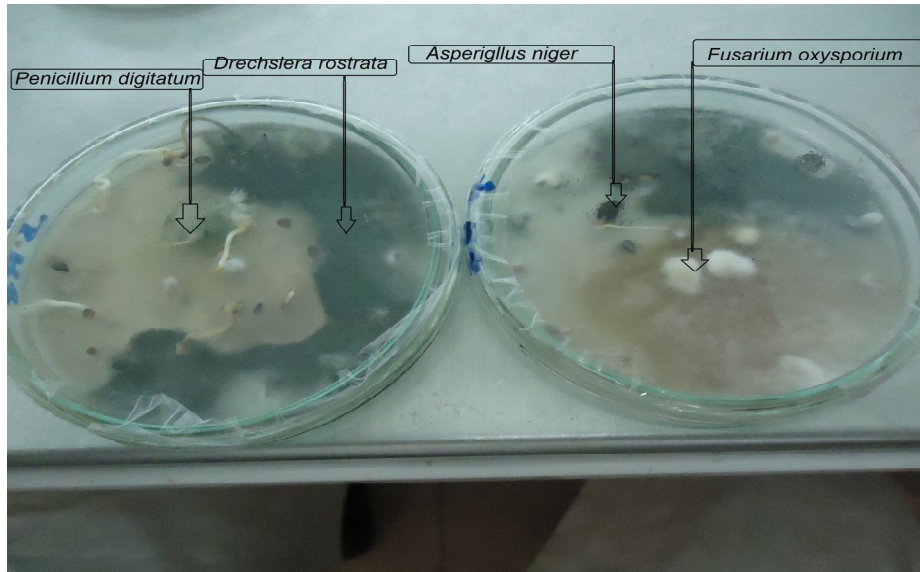


Plate (9) fungi detected on Agar:

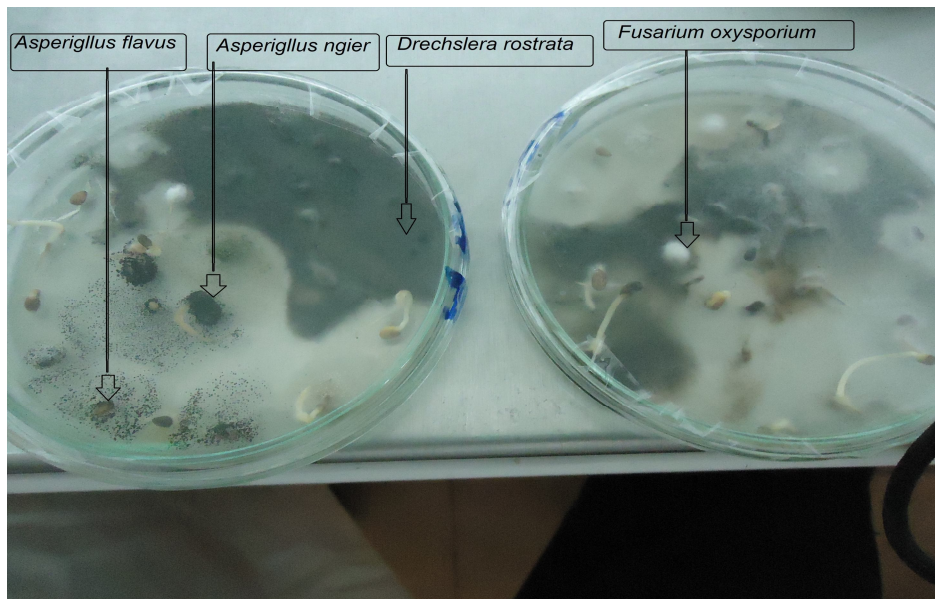


Plate (10) fungi detected on Agar



Plate (11) *Drechslera rostrata* Magnification (x40):

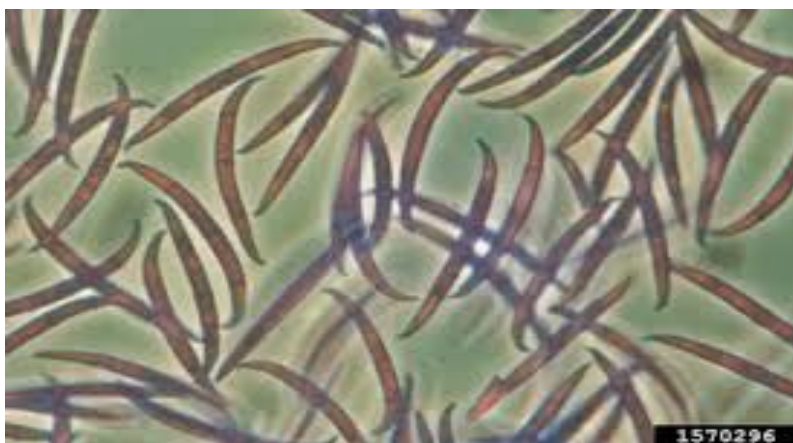


Plate (12) *Fusarium oxysporum* Magnification(x40):



Plate (13) *Alternaria sesami* Magnification(x40):

4.2 Control:

This study which conducted under laboratory condition of plant pathology, College of Agricultural Studies, Sudan University of science and Technology during the period September to December 2013 to investigate the inhibitory effect of Neem and Damas leaves aqueous extracts and fungicide, Tilt 250 EC efficacy against the fungus *Drechslera rostrata*.

The results (Table4and Figure 1) showed that the leaves aqueous extract of Neem. Neem was effect on the fungal growth at (10%) gave significantly higher inhibition zones percent (87.6%, 81.6%and 81.4%) respectively compared to the untreated control, and the results (Table 5and Figure 2) showed that the leaves aqueous extract of Damas. Damas was effect on the fungal growth at (15%) gave significantly higher inhibition zones percent (81.3 %, 82.and 83.8) respectively compared to the untreated control. And results (Table 6 and Figure3) showed that the Fungicides Tilt. The Tilt was best effect on the fungal growth at(5%) gave significantly higher inhibition zones percent(93.2%,90.4and 86.1%) respectively compared to the untreated control.

Table (4) Effect of plant leaves extracts of Neem on the growth of linear *Drechslera rostrata* in vitro at % of inhibition

Conc. (%)	Inhibition zone (%)			
	Time (day)			
	2 days	4days	6 days	7 days
5%	70.4 (8.3) a	81.1 (9.0)a	87.1(9.3)a	87.6 (9.3)a
10%	88.2 (9.3)a	80.6 (9.0)a	88.1 (9.4)a	81.6 (9.0)a
15%	70.6 (8.8)a	78.6 (8.9)a	88.3 (9.4)a	81.4 (9.0)a
Control	0.0 (0.7)b	0.0 (0.7)b	0.0 (0.7)	0.0 (0.7)b
SE	0.5			

Any two mean value (s) bearing different superscripts (s) are differing Significantly ($p < 0.05$).

❖ Data in parentheses transformed using square root transformation $\sqrt{X + 0.5}$ before analysis

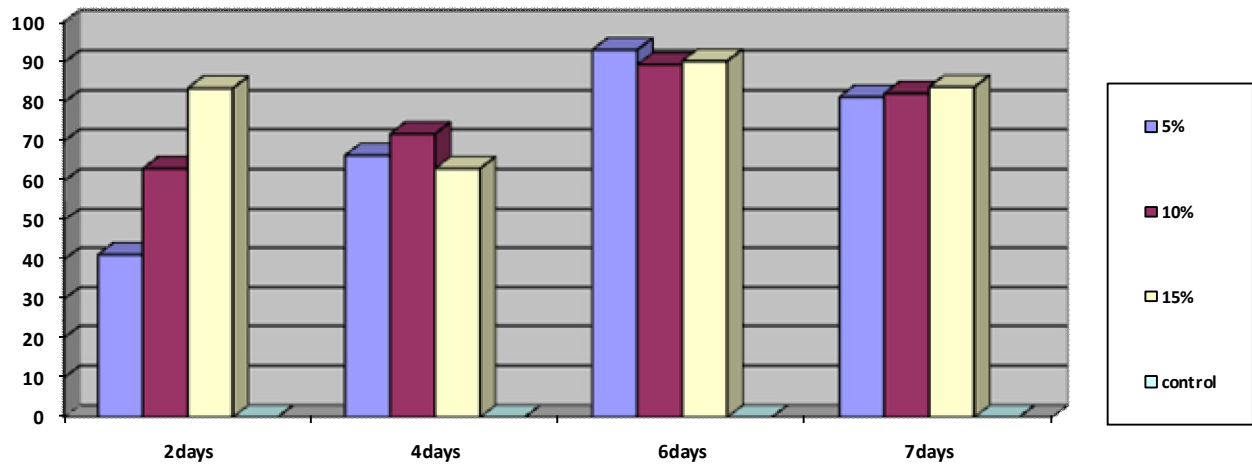


Figure (4) Effect of plant leaves extract Neem on the linear growth of *Drechslera rostrata* in vitro.

Table (5) Effect of plant leaves extracts of Damas on the linear growth *Drechslera rostrata* in vitro:

Conc. (%)	Inhibition zone (%)			
	Time (day)			
	2 day	4days	6 days	7 day
5%	41.2(6.4)c	66.4(7.5)a	93.1(9.6)a	81.3(9.2)a
10%	63.1(7.9)b	71.8(8.5)a	89.4(9.4)a	82.2(9.1)a
15%	83.5(9.1)a	63.1(8.0)a	90.3(9.5)a	83.8(9.2)a
Control	0.0 (0.7)d	0.0 (0.7)d	0.0 (0.7)d	0.0 (0.7)d
C.V	9.9			
SE	0.4			

Any two mean value (s) bearing different superscripts (s) are differing significantly ($p < 0.05$).

❖ Data in parentheses transformed using square root transformation $\sqrt{X + 0.5}$ before analysis

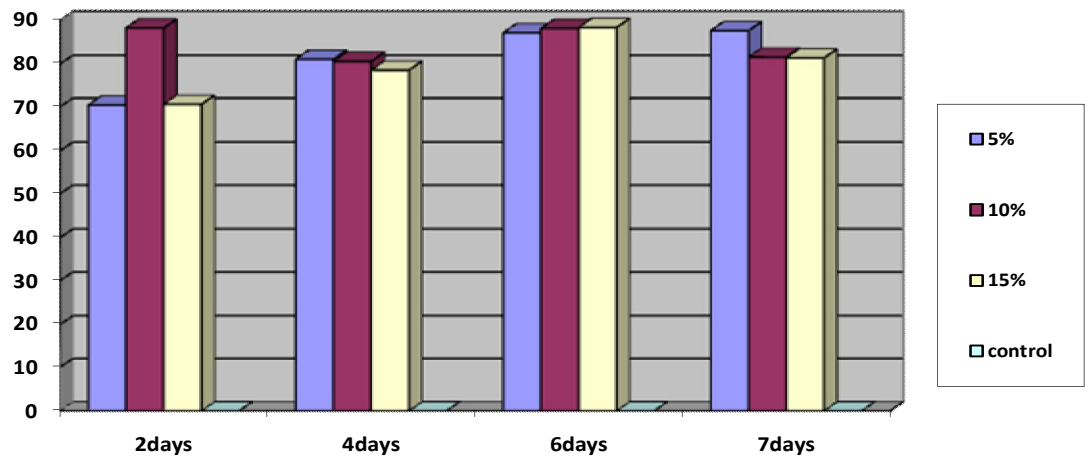


Figure (5) Effect of plant leaves extract Damas on the linear growth of *Drechslera rostrata* in vitro

Table (6) Effect of Tilt on linear growth of *Drechslera rostrata* in vitro:

Conc.	Inhibition zone (%)			
	Time (day)			
	2 day	4days	6 days	7 day
5%ppm	82.6 (9.0)a	91.6(9.6)a	81.1(9.0)a	93.2(9.6)a
10%ppm	51.5 (7.2)b	81.8(9.0)a	86.1(9.3)a	90.4(9.5)a
15%ppm	38.2 (7.6)b	89.1(9.4)a	88.1(9.4)a	86.1(9.3)b
Control	0.0 (0.7)c	0.0 (0.7)c	0.0 (0.7)c	0.0 (0.7)c
C.V	17.5			
SE	0.25			

Any two mean value (s) bearing different superscripts (s) are differing significantly ($p < 0.05$).

❖ Data in parentheses transformed using square root transformation $\sqrt{X + 0.5}$ before analysis.

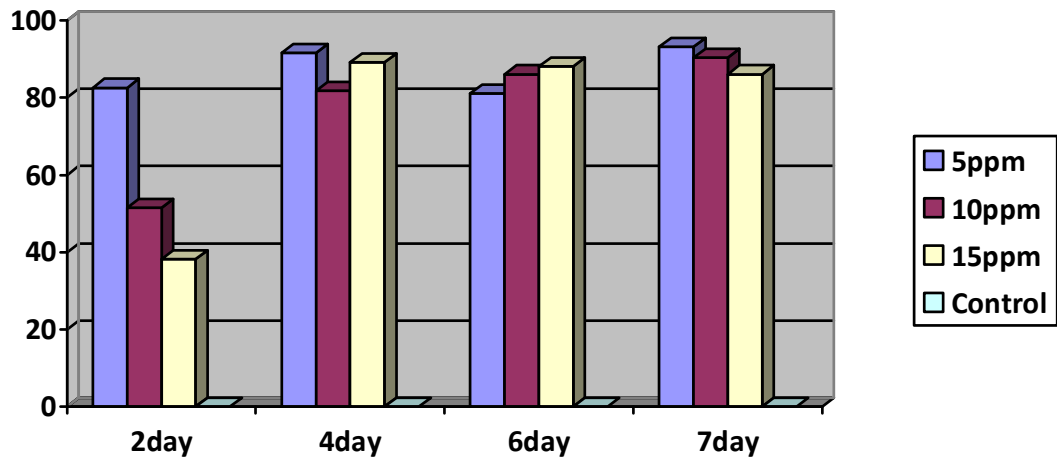


Figure (6) Effect of Tilt on the linear growth *Drechslera rostrata* in vitro:

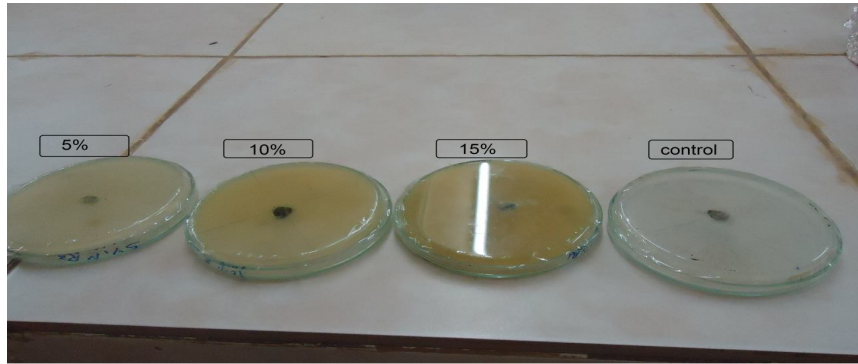


Plate (14) Effect of Neem Extract on linear growth of *Drechslera rostrata* in vitro:

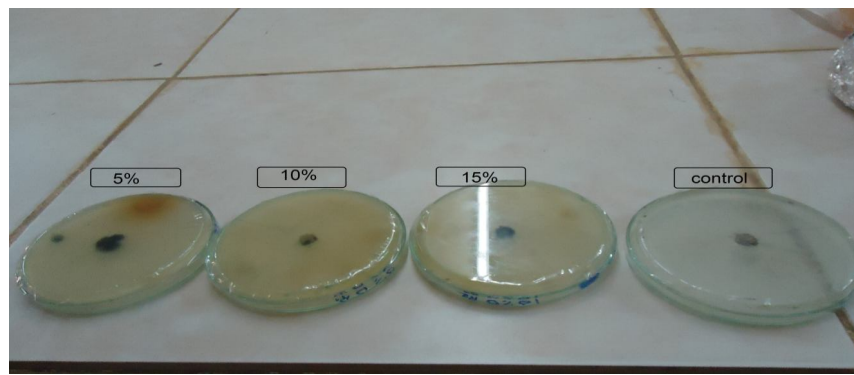


Plate (15) Effect of plant Damas Extract on linear growth of *Drechslera rostrata* in vitro:

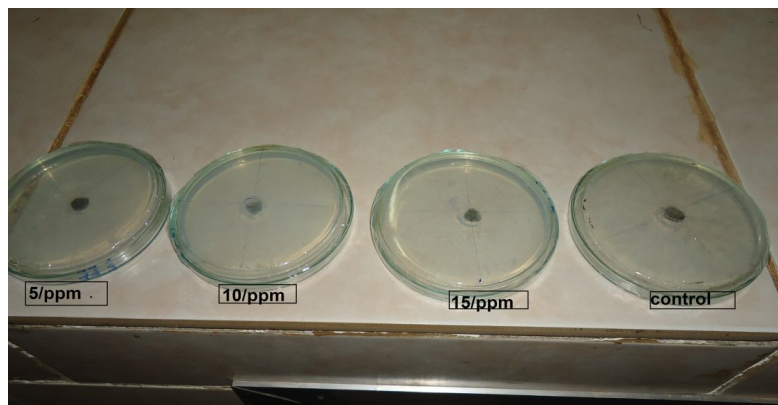


Plate (16) Effect of tilt fungicide on linear growth of *Drechslera rostrata* in vitro:

4.3 Pathogenicity test

In the exponent of the pathogenicity the disease incidence of the fungus detected.

In pre –emergence result was Gadarif (21) death of the seeds, Jazera (18) death of the seeds and Radoom (22) death of the seeds .Radoom was best of the compared to the healthy seedling and post- emergence result was Gadarif (12) damping off, Jazera (12) damping and Radoom (11) damping off. Radoom was best of the compared to the healthy seedling. As show in the table (7), Figure (5), plate (15).

Table (7) incidence in pre- and post- emergence seedlings compared to healthy seedlings:

<i>Sample</i>	<i>No. of seeds sown</i>	<i>Pre – emergence</i>	<i>Post – emergence</i>	<i>Healthy Seedling</i>
Gadarif	40	21	12	7
Jazera	40	18	12	10
Radoom	40	22	11	7

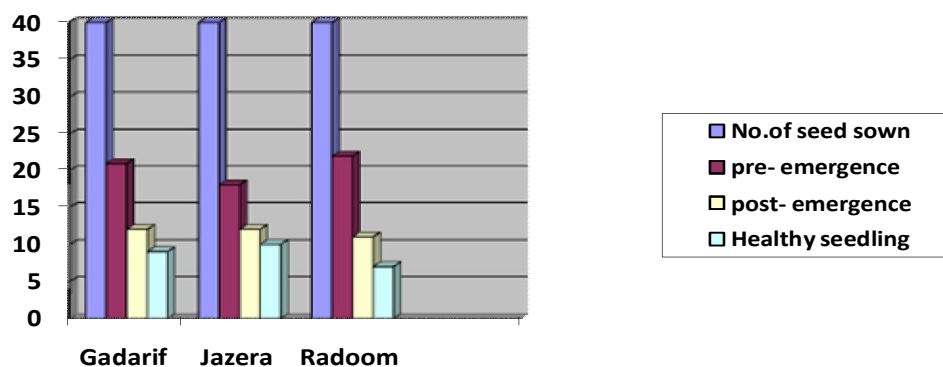


Figure (7) incidence in pre- and post- emergence seedlings compared to healthy seedlings:



Plate (17) Pathogenicity test of Drechslera rostrata, Fusarium oxysporum and Asperigllus ngier A. flavus in soil:

CHAPTER FIVE

DISCUSSION

In the present work, dry inspection for three cultivars (Gadarif, Jazera and Radoom Beladi) *Sesamum indicum* L. The result revealed the percentage of pure healthy and unhealthy, broken, weeds, discoloured and malformed seeds. Gardarif was best percentage of healthy is 71%, 70% in Radoom Beladi and 65% in Jazera and unhealthy percentage 29% in Gardarif, 29.5% in Radoom Beladi and 35% in Jazera. This result agrees with conducted according to (ISTA Rules 1966). Seeds of three sesame cultivars namely were (Gadarif, Jazera and Radoom Beladi) were associated with 6 species of fungi *Derchslera rostrata*, *Fusarium oxysporum*, *Alternaria sesami* *Asperigllus ngier*, *Asperillus flavus* and *Penicillium digitatum*. This result closely agrees with the result of (Noble Richardson (1968), Mathur and Kabeere (1975), (Ershad Riahi2000).

Derchsletra rostrata was detected in high percentage range 21%, 17.5% and 17.5% on Blotter and 22%, 18.5% and 19% on Agar plate method. These results agree with Salih (1985) who has shown that is seed-borne fungi were encountered in 25-seed samples of sesame and reported that *Derchsletra rostrata* was detected on several tropical and temperate crops among 26 species of *Derchsletra* observed on seed (Chidambaram1969-1971).

Fusarium oxysporum was detected in the range 12.5%, 14.5% and 15% on Blotter and 17.5%, 15% and 15% on Agar plate method. This result agrees with Mathur and Kabeere (1975), Leppuk and Sowell (1964), (Noble Richardson1968).

Alternaria sesami was in the range 11%, 12% and 10% on Blotter and 14%, 9% and 13% on Agar plate method. This result agrees with Leppuk and Sowell (1964), Mathur and Kabeere (1975), Wu (1988).

Asperigillus ngier and *Asperigillus flavus* were detected in the range 6.5%, 11.5%, 6.5%, 8%, 6.5% and 8% on Blotter and 10%, 10%, 6.5%, 8.5%, 6.5% and 10% on Agar method. This result agrees with (Kamees and Schlosser 1990).

Penicillium digitatum was detected in the range 10%, 6% and 10% on Blotter and 7.5% and 7.5% on Agar method. This result agrees with (Ershad Riahi 2000).

The effect of plant extracts (Neem, Damas) and fungicide (Tilt) suitable for the control of *Derchsletra rostrata* in vitro investigated. Neem tree (*Azadirachta indica*) as well is one of the most known plants for its multiuse in controlling insect pests and diseases. In this study the results revealed that the Neem leaves aqueous extracts suppressed the fungal growth with significantly high inhibition zones percent compared to control (Tables 4 and Figures 4) and study also demonstrated that the Damas leaves extract exhibited more inhibitory effect than that of the Neem. This could be attributed to the high concentration of the bioactive inhibiting compound in the Damas plant leaves than in the Neem. Moreover, the data on concentrations from each plant leaves aqueous extract exhibited different inhibitory abilities on fungal growth. Neem was no growth observed at 10% concentration and Damas was where no growth was observed at 15% concentration. Similar studies which explored the effect of extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials (Satish et. al., 1999; Okigbo and Ogbonnaya, 2006;

Ergene et. al., 2006; Kiran and Raveesha, 2006; Mohana and Raveesha, 2006).

The results on effect of the Tilt on the fungus showed that the fungicide at 5% concentrations expressed suppressive ability on the growth of the *Fusarium* with significantly high inhibition zones percent compared to control (Tables 6 and Figures6), this result was coincided with (Tahani 2005).

In the pathogenicity test sesame was mixed with spore suspension of the fungi (*Derchsletra rostrata*, *Fusarium oxysporum* and *Asperigllus flavus*). That reveled Death of the seeds and Damping off of seedling was observed at 5days. This result was similar result found by (Jamal. 2006).

RECOMMENDATIONS

- 1- More seed samples of Sesame have to be tested to get a good survey for seed-borne pathogens from different parts in the Sudan.
- 2- More biological investigation is recommended for control of seed-borne pathogens such as argil, Usher for Damas.
- 3- Improvement of storage conditions for seed in production schemes.
- 4- Growing on test is recommended to follow disease cycle from seed to seed with emphasis on damping-off pathogens in pre- and post- emergence of seedling this is complementary seed quarantine.

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APPENDIXS

(a) Potato dextrose agar (P D A) Medium :- Components

Agar	20g
Dextrose	20g
Distilled water	1000ML.

(b) Powder of plant Extract (Neem, Damas) Components.

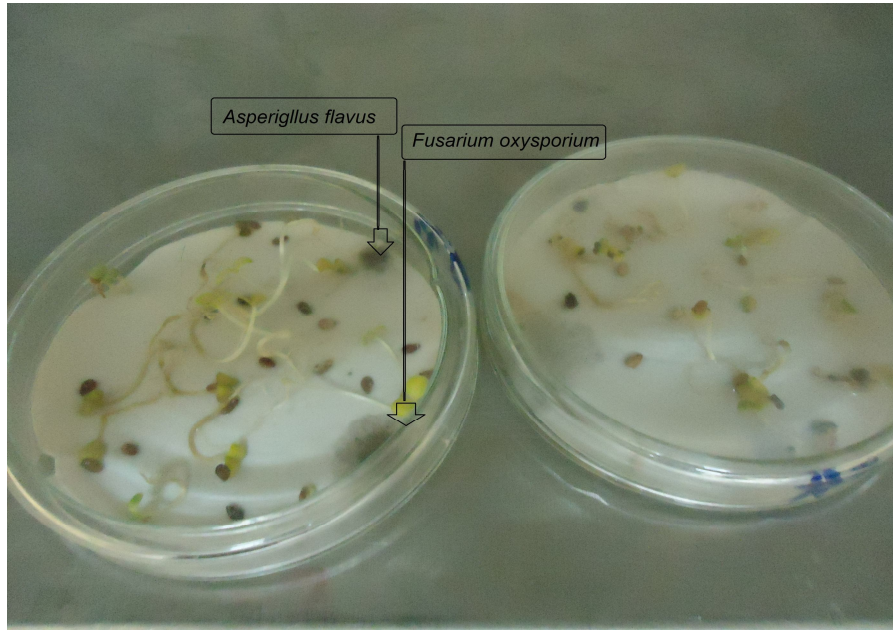
Powder of (Neem)	Distilled water
5g	95ml.
10g	90ml.
15g	85ml.

Powder of (Damas)	Distilled water
5g	95ml.
10g	90ml.
15g	85ml.

(c) Fungicide (Tilt) Components.

Tilt	Distilled water
5ppm	95ml.
10ppm	90ml.
15ppm	85ml.

(d) Plate fungi detected on blotter.



(e) Table 4: Analysis of variance table (One way ANOVA table):

Degrees of Freedom	Sum of Square	Mean Square	F- Value	Prob.
Between 3	149.630	49.877	62.650	0.000
Within 8	4.307	0.538		
Total 11	153.937			

Coefficient of Variation = 10.8%

(f) Table 5: Analysis of variance table (One way ANOVA table):

Degrees of Freedom	Sum of Square	Mean Square	F- Value	Prob.
Between 3	125.927	41.976	114.219	0.0000
Within 8	2.940	0.363		
Total 11	128.867			

Coefficient of Variation=9.9%

(g) Table 6. Analysis of variance table (One way ANOVA table).

Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between 3	123.380	41.127	35.582	0.00000
Within 8	9.247	1.156		
Total 11	132.627			

Coefficient of Variation = 17.53