SOME STUDIES ON THE FUNGUS *Macrophomina phaseolina* (Tassi) Goid ISOLATED FROM CHICKPEA (*Cicer arietinum* L.) SEEDS

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DEDICATION

To my father,

To my mother, brothers and sisters,

To my son and husband,

With great love and respect to all who helped

And to friends

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My unlimited thanks are to Alla, who offered me health and strength to do this work.

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ABSTRACT

This research was conducted on four varieties of chickpea seeds to find out the fungi that were carried on or inside those seeds. One variety was brought from Zalingi another from Jebel Marra and the other two from Hudeiba Research Station (Shendi, Wad Hamid).

The tests were performed according to (ISTA Rules, 1966) which included blotter test, agar plate method and dry seed inspection. The dry seed inspection revealed contaminated, malformed and broken seeds. After the seeds were treated with sodium hypochlorite 1% solution the following fungi were found: *Macrophomina phaseolina*, *Drechslera* sp. and *Alternaria* sp. *M. phaseolina*, was detected in high percentage. The fungus was isolated and the tests performed showed that malt-extract was the best medium for the growth of the fungus. When the fungus was treated with the fungicide Bayleton it has reduced its growth even when used in the low concentration of 10 ppm. On the other hand the fungicide Tilt has completely inhibited the growth of the fungus.

CHAPTER ONE

INTRODUCTION

CHICKPEA (*Cicer arietinum* L.) belongs to the family Fabaceae. This includes the grain legumes (pulses) which play an important role in meeting the quantitative and qualitative protein requirements of a large part of humanity, especially in the developing countries of Asia, Africa and Latin America. They also fix large quantities of atmospheric nitrogen. Of the grain legumes, chickpeas stand second in area occupying 23 million feddans and third in quantity (7 metric tons). More than 85% of the area, as well of production, is India (Simmonds, 1976).

Seeds of chickpea are eaten fresh or as cooked dry pulse, parched or boiled. Sprouted seeds are eaten as a vegetable or salad. Young plants and green pods are eaten like spinach. Dhal is the split chickpea without its seed coat dried and cooked into a thick soup or ground into flour for snacks and sweet meat. Leaves are used for livestock feed, whole seeds may be milled directly for feed for horses and cattle. Leaves are said to yield an indigo like dye. Acid exudates from the leaves can be applied medicinally or used as vinegar. Chile, a cooked chickpea milk mixture (4:1) was good for feeding infants, effectively controlling diarrhea.

In Sudan, chickpea ranks third in consumption among cool season food legumes, being preceded by faba beans and lentils. Most of the chickpea grown in Sudan is produced under river flood, although its cultivation with pump irrigation is expanding. The total cultivated area in Sudan is about 200 feddans, producing an average yield of 247.52 kg/feddan (FAO, 1987). In good years of river flood, the area under chickpea cultivation in Sudan may reach 8568 feddans (Faki *et al*., 1992). The major producing areas in the country include the Northern State, Jebel Marra region and river Rahad district (Faki *et al*., 1989). A major biotic constraint to the productivity of chickpea is the damage to the crop caused by diseases, insect pests, nematodes, and parasitic weeds. These organisms are also responsible to a large extent, for instability in the yield of the crop in the major production areas in the world (Saxena and Singh, 1986).

The main fungi that affect chickpea are *Fusarium oxysporum* (= orthoceras) *F. ciceri,* causing plant wilt from about 1 month after sowing onwards, and *Ascochyta* (*Mycosphae reila*) (Phyllosticta). Rabie blight is the most serious disease in North India, Pakistan, and Middle East (sometimes causing 100% losses). Other fungi known to attack grains include *Alternaria* sp., *Ascochyta, Botrytis cinerea, Fusarium solani, Rhizoctonia bataticola, Rhizoctonia solani,* …etc.

The main objectives of this study were to:

1. Investigate seed borne fungi associated with chickpea grown in Jebel Marra, Zalingi and Hudeiba.
2. Investigate the effect of the two fungicides (Bayleton and Tilt) on *Macrophomina phaseolina*.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 Chickpea

Cicer arietinum L.

Family: Fabaceae

Common Names: Chickpea, Bengal gram, Dhal, Garbanzo, Gram, Poischicke.

Cicer belongs to the tribe Viciae. It is a relatively small genus, with 39 known species distributed mainly in central and western Asia. It has been suggested that *Cicer* exhibits a reticulate combination of characters of *Vicia* and of the genus *Ononis* of the tribe Trifoliae (Maesen, 1972). The most common somatic chromosome number reported in the genus so far zn = 2x = 16.

2.2 Recent History

Considered on global scale, there has been little variation in the area cropped with chickpea during the last two decades. Productivity has shown some slight improvement, as has total production. The relative stagnation can be traced to the fact that little attention has been paid, recently, to the improvement of the crop by breeding. It has always been considered as a poor man’s food and grown under conditions of limited inputs where other

crops can not be grown. However, substantial breeding efforts have been made in India and in Pakistan in the past two or three decades.

2.3 Prospects

The world-wide shortage of protein foods and the energy crisis leading to fertilizer shortage has fostered a new interest in grain legumes, including chickpea, and there has been considerable international concern. It is likely, therefore, that there will be greater efforts both national and international, for the improvement of chickpea than in the past. The major objectives may be to develop a plant type, with high yield potential and responsive to agronomic inputs, which can compete on a more nearly equal footing with high yielding cereals.

2.4 Germplasm

Many “varieties” have been described, but are not widely recognized. Improved cultivars have been developed, often for local adaptation. Flower and seed color and size, growth duration, yield, and disease resistances vary. Cream seeded or ‘Kabuli’ chickpea (Mediterranean and Middle Eastern origin) have the largest seeds and grow well under irrigation. ‘Desi’ chickpea (Indian distribution) have smaller seeds and yield better in India and often elsewhere. Hybrids between Kabuli and Desi have produced strain with medium-size seeds and fair yields.

2.5 Diseases of *Cicer arietinum* and their Control

Ascochyta blight, *Rhizoctonia* root rot, *Pythium* rot, *Fusarium* wilt, white mold and bacterial blight diseases are typical diseases which affect other pulses or legume crops, accelerated by periods of high rainfall, high humidity and high temperature. These are best controlled by using good seed quality, proper crop rotation, proper tillage practice, burning crop residues and disease resistant varieties are used if available (Kaminski, 1982).

2.6 Insect and Other Predators and their Control

Although chickpea leaves, stems and pods are heavily pubescent with glandular hairs that secrete malic and oxidic acids, they suffer little direct damage from aphids and other insects. Several viral diseases transmitted by aphids have occasionally been reported in chickpea fields. Seed corn maggots and wine worms might be expected to cause problems early in season by attacking the germinating seeds and destroying the growing points Eschulte and Doll (1982).

2.7 The Fungus *Macrophomina phaseolina* (Tassi.) Goid

2.7.1 Taxonomic Position

Kingdom: Fungi

Phylum: Mitosporic fungi

Class: Coelomycetes

2.7.2 Other Names Used

*Macrophomina phase*oli (Maubl.) S.F. Ashby

Sclerotium bataticola Taubenh.

Macrophoma phaseoli Maubl.

Macrophoma cajani Syd., P. Syd. And E.J. Butl.

Macrophoma phaseolina Tassi.

Macrophomina philippines Petr.

Macrophoma corchori Sawada.

Macrophoma sesame Sawada.

Rhizoctonia lamellifera Small.

Dothiorella cajani Syd., P. Syd. And E.J. Butl.

Botryodiplodia phaseoli (Maubl.) Thirum.

Rhizoctonia bataticola (Taubenh.) E.J. Butler (Anamorph).

2.7.3 Common Names

- English

  Charcoal rot of bean/tobacco

  Blight of bean/tobacco

  Ashy stem decay of bean/tobacco

  Ashy stem blight

  Root rot of bean/tobacco

- USA

  Dry weather wilt

  Summer wilt

2.7.4 Host range

Primary hosts: *Nicotiana tabacum* (Tobacco), *Phaseolus* (beans), *Solanum melongena* (aubergine), *Arachis hypogae*a (groundnut), *Zea mays* (maize), *Glycine max* (soyabean),*Sorghum bicolor* (sorghum), *Helianthus annuus* (sunflower), *Phaseolus vulgaris* (Kidney bean), *Corchorus olitorius* (nalta jute), *Lens culinaris* spp. *culinaris* (lentil), *Capsicum annuum* (bell pepper), *Cicer arietinum* (chickpea), *Gossypium barbadense* (gallini cotton), *Vigna unguiculata* (cowpea), *Vicia faba* (broad bean), *Brassica*, *Abelmoschus esculentus* (okra), *Pisum sativum* (pea), *Cajanus cajan* (pigeon pea), *Solanum tuberosum* (potato), *Oryza sativa* (rice), *Carthamus*

tinctorius (safflower), *Sesamum indicum* (sesame), *Vigna mungo* (black gram), *Vigna radiate* (mung bean), *Flaeis guineensis* (African oil palm), *Allium cepa* (onion), *Psidium guajava* (guava), *Cocos mucifera* (coconut), *Piper bette* (betel pepper), *Ricinus communis* (castor bean), *Pelargonium* (pelargoniums), *Linum, Jasminum* (jasmine), *Cichorium* (chicory), *Trigonella foenum-graecum* (fenugreek), *Curcumal longa* (turmeric), *Actinidia chinensis* (Chinese gooseberry), *Cucumis me*lo (melon), *Lycopersicon esculentum* (tomato), *Daucus carota* (carrot), *Ables concolor* (white fir), *Pseudotsuga menziesii* (Douglas-fir), *Cedrus atlantica* (Atlas cedar), *Cetrus deodara* (Deodar cedar), *Lagenaria siceraia* (bottle gourd), *Vagna aconitifolia* (moth beans), *Parthenium argentaum* (Guayule), *Carica papaya* (Papaw), *Beta vulgaris* var. *saccharifera* (sugar beet), *Prosopis jutiflora* (Kiawa), *Stylosanthes piper nigrum* (black pepper), *Cucumis sativus* (cucumber), *Critrullus lanatus* (water melon), *Momordica charantia* (bitter gourd), *Pinus lambertina* (sugar pine), *Pinus jeffreyi* (Jeffrey pine), *Pinopsida* (conifers), *Eucalyptus tereticornis* (forest red gum), *Eucalyptus camaldulensis* (river red gum), *Saccharum officinarum* (sugar cane), *Papaver somniferum* (Opium poppy), *Citrus reticulate* (mandarin), *Sterculua urens, zingiber* (ginger), *Lablab purpureus* (lablab), *Crotalaria juncea* (sunn hemp), *Syzygium samarangense* (water apple), *Lupinus*

(lupins), *Alhagi pseudalhagi* (camel-thorn), *Cyamopsis tetragonoloba* (cluster bean), *Pinus calausa* (sand pine), *Cucurbita pepo* (ornamental gourd) *Albizia lebbeck* (Indian siris), *Crocus sativus* (saffron), *Pinus pinea* (stone pine), *Pinus canariensis* (canary pine), *Pinus pinaster* (maritime pine), *Pinus halepensis* (Aleppo pine), *Medicago* (medic), *Trifolium alexandrinum* (Berseem clover), *Coriandrum sativum* (coriander), *Raphanus sativus* (radish), *Pinus radiate* (radiate pine), *Pennisetum glaucum* (pear millet), *Mangifera indica* (mango), *Juniperus virginiana* (coast juniper), *Juniperus scopulorum* (rocky mountain juniper), *Brassica oleracea* var. *botrytis* (cauliflower), *Macrotyloma uniflorum* (horsefram), *Catharanthus roseus* (pink periwinkle), *Fagopyron*, *Fragaria*, *Prunus armeniaca* (apricot), *Prunus persica* (peach), *Prunus cerasus* (sour cherry), *Boechmeria nivea* (ramie), *Bombax malabaricum* (silk cotton tree), *Allium sativum* (garlic), *Quercus* (Oaks), *Basella alba* (Malabar spinach), *Impatiens balsamina* (garden balsam), *Vitis*, *Psophocarpus tetragonolobus* (winged bean), *Dahlia Datura stramonium* (Jamestown – weed), *Solidago canadensis* (Canadian goldenrod), *Narcissus* (daffodil), *Ipomoea batatus* (sweet potato).

2.7.5 Biology and ecology

The main inoculum source of *M. phaseolina* for a broad range of hosts is small, black micro-sclerotia in soil and host crop debris. In soybean fields in Missouri, USA, micro-sclerotia population were related to the number of years of plants maize and soybean (Short *et al*., 1978).

In soyabean fields, the survival and germination of sclerotia of *M. phaseolina* is favoured by dry soils (Dhingra and Sinclair, 1974; Gangopadhyay *et al*., 1982), high C:N ratios of amendments (Dhingra and Sinclair, 1974), low bulk density (Gangopadhyay *et al*., 1982) and oxygen concentrations greater than 15% (Todd *et al*., 1987). *M. phaseolina* survived as sclerotia in maize and sorghum stalk residues for 18 months and 15 months, respectively. Although sclerotial germination decreased during the sampling period, large numbers of viable sclerotia were isolated from each maize and sorghum stalk in the final collections (Cook *et al*., 1973). Data from a study of *M. phaseolina* isolated from jute suggested poor saprophytic (ability of *M. phaseoli* in mycelial form and the involvement of dormant sclerotia in the survival of the organism in soil (Bhattacharya and Samaddar, 1976).

Populations of the sclerotia of *M. phaseoli* in soil were directly related to the severity of charcoal rot which, in turn, was inversely related to

soyabean yields (Short *et al*., 1978). The incidence of charcoal rot in sorghum in India was positively correlated with soil populations of *M. phaseolina (r = 0.53, p = 0.01) (*Gray *et al., 1991).* Germination was reduced in two out of three sesame cultivars when seeds were placed in plastic containers filled with autoclaved soil and covered with soil containing sclerotia of *M. phaseolina* at 1000 sclerotia per gram of soil. Plant mortality, especially of 50 and 60-day-old seedlings, was higher at 333 and 500 sclerotia per gram of soil than at 200 sclerotia per gram of soil (Pineda *et al*., 1985). In sunflower fields, the inoculum density of sclerotia was greater in areas where the plants were most diseased and it was also related to crop rotation (Alabouvette, 1976). In a study in India, percentage infection in four crops tested increased linearly with an increase in the inoculum density of sclerotia in the soil, but varied inversely with soil moisture. It was consistently higher at 30 days at 15 days during the experimental period. Susceptibility varied among the test species 50% infection was achieved in *Vigna mungo* with five sclerotia per gram of soil; in *Abelomoschus esculentus* and *Cyamopsis tetragonoloba* with 20 sclerotia per gram of soil, and in cotton with 40 sclerotia per gram of soil. Inoculum densities of up to 40 sclerotia per gram of soil coupled with high soil

moisture did not produce more than 50% infection in any crop (Sheikh and Ghaffar, 1979).

M. phaseolina was recovered form the radicles of a few soyabean seedlings that germinated in autoclaved soil, but the seedlings quickly died (Gangopadhyay *et al*., 1970). Of 62 accessions of *Phaseolus vulgaris* grown in sterilized sand, the seeds of six exhibited typical symptoms of charcoal rot on emerged seedlings ranging from 5-30% (Abawi and Pastor Corrales, 1990). There is no other evidence for seed transmission of the pathogen in any crop.

The process of infection after inoculation with *M. phaseolina* has been studied in several hosts. Necrotic patches were observed on root 42 h after inoculation of sorghum. Hyphae penetrated directly within 24 h and extended inter-and intracellularly into the epidermals cortical and vascular tissues. Disintegration of xylem parenchyma, phloem and cortical cells was observed within 3 days. Sclerotia necrotic by the sixth days were formed within the host tissues after colonization. Most of the cortical cells observed were necrotic by the sixth day and, as a result, hyphae could not be distinguished. By the eighth day sloughing-off of nectrotic material from the cortex was noticed (Karunakar *et al*., 1992). The presence of germinating sesame seeds and seedlings stimulated normal sclerotial

germination and attraction of developing mycelium on the host roots. Infection cushions and appressoria were also formed prior to infection (Abdou *et al*., 1979).

Inoculum, present in soil, colonized jute as soon as it came into contact with it, and hyphal growth followed the lines of junction of under - lying epidermal cells.

Penetration of the epidermis took place at the junction of two epidermal cells the lateral cell walls of the epidermis and the cell walls of cortical cells of diseased tissues did not show the positive reaction for pectin seen in corresponding healthy tissues. Cells of the cortex and phloem failed to stain for cellulose in diseased tissues infected cortical tissues, cambium and epidermis were stained intensely with schiffs reagent.

Roots of chickpea inoculated with *M. phaseolina* showed disintegration of cortical tissues, while mycelium and sclerotial bodies were seen plugging the xylem in longitudinal sections. The sudden collapse of infected plants at the pod-filling stage may be due to this plugging of the xylem vessels (Singh and Nene, 1990). Infection of cowpeas (grown in steam-pasteurized soil drenched with suspensions of *M. phaseolina* microsclerotia before planting) occurred underground in emerging cotyledons and hypocotyls and roots were colonized completely but

appeared healthy. No evidence was found for internal growth of the fungus from the cortical lesions or from the roots, and mycelium was not detected in microscopic examinations of stem pith, phloem and xylem (DeMooy and Burke, 1990).

The severity of disease by *M. phaseolina* in various hosts is associated with warm dry conditions. In India epiphytotics occur in soyabean in areas where temperatures range from 35 to 40°C, and controlled environment experiments have shown maximum infection occurring in inoculated seedlings grown at 30-40°C (Agarwal *et al*., 1973; Meyer *et al*., 1974). In three field experiments on sorghum, plants subjected to post-flowering water stress and inoculated with *M. phaseolina* had greater development of charcoal rot symptoms than inoculated plants not subjected to water stress (Diourte *et al*., 1995). Under field conditions, disease intensity increased in sesame with a progressive rise in temperature and decrease in RH. Maximum disease occurred at 35°C and 76% RH (Patel and Patel, 1990). Water and temperature stress have also been implicated in this disease for sunflowers (Tosi and Zazzerini, 1990), maize (Singh and Kaiser, 1992), musterd (Srivastava and Dhawan, 1979) safflower (Siddaramaiah and Hegde, 1982), jute (Cheng and Tu, 1972),

Phaseolus vulgaris (Magalhaes *et al*., 1982) and *Vigna radiate* (Indra-Hooda *et al*., 1990).

Increased infection of a range of hosts by *M. phaseolina* occurred when the fungus was co-inoculated with nematodes. A synergistic relationship with *M. phaseolina* has been found for *Heterodera glycines* on soyabean (Short *et al*., 1980), *Meloidogyne incognita* on several hosts (Short, *et al*., 1980; Sharma, *et al*., 1980; Al-Hazmi, 1985; Mishra, *et al*., 1988; Hussain, *et al*., 1991) and *Rotylenchulus reniformis* on lentil (Anver, *et al*., 1991). Adults of the insect *Cylindrocoptunus adspersus* carry *M. phaseolina* externally and transmit the pathogen to sunflower during oviposition via the sealed egg cavity in the stalk (Yang *et al*., 1983).

Physiological races or strains have not been identified for *M. phaseolina*. Nuclear and chromosomal patterns provide the fungus with the ability to adapt to a variety of environmental conditions (Todd *et al*., 1987). This has been evidenced by differences in virulence and cultural characteristics for isolates from different parts of soyabean plants (Chitima-Matsiga and Wyllie, 1987). Ten isolates from India rapidly developed tolerance to several fungicides after several transfers through fungicide – amended plates (Oyekan and Naik, 1987). Isolates of *M. phaseolina* from other hosts have also shown similar differences in virulence and cultural

characteristics, including those from sesame (Simosa and Delgado, 1991), Sunflower (Maqbool – Ahmed, *et al*., 1992), Groundnut (Sobti and Bansal, 1988), Chickpea (Singh and Nene, 1990), Vigna mungo (Vidhyasekaran and Arjunan, 1978) and Cotton (Monga, *et al*., 1994).

2.7.6 Symptoms

Chickpea

(i) **Seedlings:** The first symptom appears on the stem at the soil line as a small, irregularly shaped, blackish, sunken lesion. Lesions may occur before or soon after emergence.

(ii) **Upper plant:** Infection spreads upwards from the original canker. Several cankers may enlarge, coalesce and eventually girdle and kill the plant. Cankers possess a definite margin and commonly contain concentric rings wilting, chlorosis and death of leaves may be more pronounced on one side of the plant. Numerous small, black sclerotial bodies or pycnidia from the aging, ash-grey cankers. Large areas of the crop may be killed.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Chickpea Seed Sample Supply

Four varieties of chickpea obtained from Jebel Marra, Zalengie Hudeiba, Shendi and Wadhamid were kept in paper bags (1 kg). These seeds samples were representing seed lots harvested in 2004 localities Shendi, Wad Hamid, Jebel Marra and Zanlingi and were kept in refrigerator, in the Plant Protection Directorate Laboratory.

3.2 Detection of Seed-borne Fungi Associated with Chickpea

3.2.1 Dry inspection

Four hundred seeds of chickpea randomly selected from each variety were examined with the aid of a stereoscopic binocular microscope. Observations included impurities, sclerotia, galls, discoloration and malformation.

3.2.2 Blotter test

3.2.2.1 Untreated seeds

Four hundred seeds from each sample were tested by the standard blotter method (ISTA, 1966). Each seed sample was plated in sterilized plastic Petri-dishes (9 cm diameter) on moistened three layers of blotter at

the rate of 10 seeds per plate. The seeds were placed equidistantly from each other. The plates were then incubated in a controlled environment chamber (Sohieb, 1983) at 25-30°C under alternating cycles of 12 hours near ultraviolet (NUV) light (at a distance of 40 cm) and 12 hours darkness for 7 days; detections were done on the 8th day using sterio-and compound microscopes.

3.2.2.2 chlorine pre-treated seeds

Four hundred seeds of each sample were treated with sodium hypochlorite (1% available chlorine) for 3-5 minutes. Seeds were then washed with sterilized water. Washed seeds were plated in Petri-dishes provided with filter paper. Then the experiment was carried in a laminar device and then placed in the incubator. On the 8th day the Petri-dishes were examined for the presence of fungi.

2.3.3 Agar Plate Method

In this method the media was prepared from extract of 250 g of potatoes, 20 g of dextrose and 15 g of agar. These substances were completed to 1000 ml sterile water to make one litre of the medium and autoclaved for 20 min at 15 lb pressure. The medium was then left to cool at room temperature. Then 200 seeds of each variety of chickpea were carefully surface sterilized by (1% sodium hypochloride) and washed by

sterilized water. The seeds were dried by blotters and then plated on PDA medium. The seeds incubated were 10/plate. The plates were labeled and kept in the incubator at 28°C for seven days. On the 8th day the seeds were examined by steriobinocular and compound microscope for the presence of fungi.

3.3 Optimum Medium for the Growth of the Fungi

In this test three types of media were used as follows:

3.3.1 Potato Dextrose Agar (PDA)

Potato extract (250g), Dextrose 20 g, Agar 15 g as mentioned before and then autoclaved for 20 minutes.

3.3.2 Malt Extract Agar (MEA)

Consisted of 25 g malt dissolved in 1000 ml of sterilized water and shaked and then autoclaved for 15 minutes.

3.3.3 Czapeks Dox Agar Medium

25 g of Czapeks medium were dissolved in 1000 ml of sterilized water shaken and autoclaved for 15 minutes.

The fungus was grown on PDA medium for seven days. From the edge of the medium 5 mm disc was taken by forceps and placed in each of the different media (PDA, MEA and Czapeks). The plates were incubated at 28°C for 7 days. The rate of the fungus growth was estimated daily by

measuring the colony size along the two diameters drawn on the back of each plate, then the mean of colony diameter was calculated for each medium.

3.4 Effect of Fungicides on Linear Growth of *M. phaseolina*

In this experiment two fungicides were used. These were Tilt and Bayleton. The concentrations tested for the two fungicides were 10, 30, 50 and 70 ppm.

3.4.1 Fungicide preparation

One gram of Bayleton was dissolved in 1000 ml sterilized water, and similarly one gram of Tilt was dissolved in one litre of sterilized water. From this solution one ml of the fungicide solution was poured in a flask (100 ml volume) and completed with 99 ml. MEA media shaken and put in the Petri-dishes. The fungus *Macrophomina phaseolina* 7 days old culture was then grown in this media. The same procedure was used to prepare 3 ml, 5 ml and 7 ml media. These preparations of the fungicides concentrations were tested. Four replicates of each concentration was used. The control treatment without fungicide was also replicated four times. The Petri-dishes were inoculated at 28°C. Every 24 hours the fungal growth was measured and the diameter of colony was recorded for 7 days.

CHAPTER FOUR

RESULTS

4.1 Detection of Seed-borne Fungi

4.1.1 Dry inspection

Four hundred seeds were inspected by the steriobinocular and compound microscope as well as the magnified hand lens. Results revealed the presence of sclerotia, malformed seeds and plant debris for the four cultivars tested as shown in Table 1.

4.1.2 Blotter test (untreated seeds)

This test revealed the presence of high percentage of saprophytes in non pre-treated seeds by sodium hypochlorite whereas few percentage of *Aspergillus flavus* and *Aspergillus niger* were detected as shown in Table 2.

4.1.3 Blotter test (treated seeds)

Seeds were treated with sodium hypochlorite (1%). The following fungi were observed: *Alternaria alternata, Drechslera* sp. and *Macrophomina phaseolina* (Table 2).

4.1.4 Agar test

Four hundred seeds of *Cicer arietinum* from each cultivars were tested by the agar plate method. The fungus detected was only *Macrophomina phaseolina* and found at the rate of 15% on cv. Wad Hamid

4.2 Physiological Studies

The fungus *Macrophomina* phaseolina was purified and tested for the suitable growth medium. The media tested were Potato Dextrose Agar (PDA), Czapeks Dox Agar and Malt Extract Agar (MEA).

Malt extract agar medium was the best compared to the other two media as shown in Table 3. Chemical control of *M. phaseolina* in vitro by Bayleton and Tilt in different concentrations (10, 30, 50, 70 ppm) revealed that Tilt fungicide is more effective even at the lowest concentration as shown in Table 4 and 5.

Table 1: Dry inspection test of 400 seeds for four cultivars of chickpea.

Variety	Healthy seeds	Seeds with sclerotia	Malformed seeds	Impurities and plant debris piece
Shendi	352	17	28	3
Wad Hamid	355	42	15	6
Jebel Marra	373	19	4	4
Zalingie	364	26	8	2

Table 2: Percentage incidence of fungi recorded by blotter test from seeds treated and untreated with sodium hypochlorite..

The fungus	% incidence in untreated seeds	% incidence in treated all cultivar
Aspergillus flavus	8.5	0.0
A. niger	3	0.0
Alternaria sp.	0.0	1
Drechslera	0.0	1
M. phaseolina	0.0	10.5

Table 3: The effect of 3 types of media on linear growth (cm).of *Macrophomina. phaseolina* (colony diameter)

Media / day	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**
PDA	1	2.6	3.2	4.3	6.1	6.8
Czapeks	0.6	2.3	3.9	4.9	6.1	6.3
MEA	1.4	3	4.5	6	7.3	8.4

Table 4: Effect of the fungicide Bayton on the in vitro linear growth (cm) of *Moacrophomina phaseolina*.

Dose / day	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**
Control	1	2.3	3.6	5	6	7.6
10 ppm	0	1.2	2.4	3.8	4.5	5.4
30 ppm	0	1	2	2.7	3	3.8
50 ppm	0	0.3	0.6	1	1.1	2
70 ppm	0	0.25	0.5	0.7	0.7	1.2

Table 5: Effect of the fungicide Tilt on the in vitro linear growth (cm) of *Moacrophomina phaseolina*.

Dose / Day	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**
Control	1	2.3	3.6	5	6	7.6
10 ppm	0	0	0	0	0	0

F 1,2

F3,4

plate1

plate2

Plate 3

F.5

Plate 4

F.6

Plate 5

F.7

Plate 6

CHAPTER FIVE

DISCUSSION

In the present study seed health testing for four cultivars of *Cicer arietinum* was conducted according to ISTA Rules (1966). The cultivars tested were Shendi, Wad Hamid, Jebel Marra and Zalingi These seed samples were representing seed lots harvested in 2004 in localities of Shendi, Wad Hamid, Jebel Marra and Zalingi.

This piece of research revealed the presence of *Macrophomina phaseolina* in 15% of the seeds of cv. Wad Hamid. Accordingly some physiological studies were carried out upon this fungus. *Macrophomina phaseolina* has a wide host range as reported by Short *et al.*(1978), Abdou,*et al*. (1979), Singh *et al*. (1990), Raj and Chattopadhyay (1983).

The suitable medium for fungal growth of *Macrophomina* was investigated on three different media. These were Potato Dextrose Agar (PDA), malt extract agar medium and czapek Dox Agar medium. Malt extract Agar medium was found the best. This finding coincides with Liang and Bai (1992).

Chemical control *in vitro* manifested that Tilt was more effective than Bayleton, even at low concentration (10 ppm). This result coincides

with the finding of Ohr, *et al*. (1996), Thanasoupoulas, *et al*. (1979) and Haware and Joshi (1979). This finding also coincides with Barelt (1991).

Recommendations for future work:

1. Pathogencity test by *Macrophomina phaseolina* on the four cultivars of *Cicer areitenum*, particularly cv. Wad Hamid.
2. Soil inoculation test by the fungus before sowing of seeds for the four cultivars of *Cicer arietinum*.
3. Further physiological studies on the fungus *Macrophomina phaseolina* such as the suitable temperature and pH.

REFERENCES

Abawi, G.S.; Pastor Corrales, M.A. (1990). Seed transmission and effect of fungicide seed treatments against *Macrophomina phaseolina* in dry edible beans. Turrialba, 40(3): 334-339.

Abdou, Y.A.; El-Hassan, S.A.; Abbas, H.K. (1979). Seed transmission and pycnidial formation in sesame wilt disease caused by *Macrophomina phaseolina phaseoli* (Maubl.) Ashby. Agricultural Research Review, 57(2): 63-69.

Alabouvette, C. (1976). The ecology of parasitic fungi in soil. VIII. An ecological study, by means of a selective method of investigation, of *Macrophomina phaseolina* in soil. *Annals de Phytopathologie*, 87(2): 147-157.

Al-Hazmi, A.S. (1985). Interaction of *Meloidogyne incognita* and *Macrophomina phaseolina* in a root-rot disease complex of French bean. *Phytopathologische Zeitschrift*, 113(4): 311-316.

Anver, S.; Tiyagi, S.A.; Yadav, A.; Alam, M.M. (1991). Interactions between *Rotylenchulus reniformis* and *Macrophomina phaseolina* on lentil. *Pakistan Journal of Nematology*, 9(2): 127-130.

Barelt, J.A. (1991). Some investigation on the fungus *Macrophomina phaseolina*. *Ind. Phytopath.*15: 14-17.

Bhattacharya, M. and Samaddar, K.R. (1976). Epidemiological studies on jute diseases. Survival of *Macrophomina phaseolina* (Maubl.) Ashby in soil. Plant and Soil, 44(1): 27-36.

Cheng, Y.H. and Tu, C.C. (1972). Effects of host variety, plant maturity, soil temperature, and soil moisture on the severity of *Macrophomina* stem rot of jute. *Journal of Taiwan Agricultural Research*, 21(4): 273-279.

Chitima-Matsiga, R.T. and Wyllie, T.D. (1987). Variability among single conidial isolates of *Macrophomina phaseolina* (Abstr.) *Phytopathology*, 77: 1702.

Agarwal D.K.; Gangopadhyay, S. and Sarbhoy, A.K. (1973). Effect of temperature on the charcoal rot disease of soybean. Indian Phytopathology, 26(3): 587-589.

Cook, G.E.; Boosalis, M.G.; Dunkle, L.D.; Odvody, G.N. (1973). Survival of *Macrophomina phaseoli* in corn and sorghum stalk residue. *Plant Disease Reporter*, 57(10): 873-875.

DeMooy, C.J. and Burke, D.W. (1990). External infection mechanism of hypocotyls and cotyledons on cowpea seedlings by *Macrophomina phaseolina*. *Plant Disease*, 74: 9, 720.

Dhingra, O.D. and Sinclair, J.B. (1974). Isolation and partial purification of phytotixin produced by *Macrophomina phaseolina*. *Phytopathologische Zeitschrift*. 80(1): 35-40.

Diourte, M.; Starr, J.L.; Jeger, M.J.; Stack, J.P.; Rosenow, D.T. (1995). Charcoal rot (*Macrophomina phaseolina*) resistance and the effects of water stress on disease development in sorghum. *Plant Pathology*, 44(1): 196-202; 22 ref.

Domsch, K.H. and Gams, W. (1972). Fungi in Agricultural Soils. London, Longman.

Faki, H.; Mohamed, A.I.S. and Ali, M.E.K. (1992). Adaptation of chickpea in Sudan, a paper presented at the workshop on chickpea. Adaptation in the WANA Region, ICARDA, Aleppo, Syria.

Faki, H.; Taha, M.B. and Elsarrag, G. (1989). Exploratory survey of chickpea production in the Nile province, 1988/89, presented at Annual Coordination Meeting of the Nile Valley Regional Program on cool season Food Legumes, ARC, Wad Medani, Sudan.

FAO (1987). Production Yearbook 41: 152.

Gangopadhyay, S.; Wyllie, T.D.; Luedders, V.D. (1970). Charcoal rot disease of soy beans transmitted by seeds. *Plant Disease Reporter*. 54: 1088-1091.

Gangopadhyay, S.; Wyllie, T.D.; Teague, W.R. (1982). Effect of bulk density and moisture content of soil on the survival of *Macrophomina phaseolina*. *Plant and Soil*, 68(2): 241-247.

Gray, F.A.; Mihail, J.D.; Lavigne, R.J.; Porter, P.M. (1991). Incidence of charcoal rot of sorghum and soil populations of *Macrophomina phaseolina* associated with sorghum and native vegetation in Somalia. *Mycopathologia*, 114(3): 145-151.

Haware, M.P. and Joshi (1979). Tilt fungicide for control of *Macrophomina phaseolina*. *Indian Phytopath*. 27: 236-237.

Hussain, S.; Ahmad, I.; Bumey, K. Siddiqui, S.U. (1991). The effect of some herbicides on mycelial growth of *Macrophomina phaseolina* (Tassi) Goid. *Pakistan Journal of Phytopathology*, 3(1-2): 43-45.

Hooda, I. and Grover, R.K. (1990). Environmental factors affecting control of *Macrophomina phaseolina* by fungicides on mung bean. *Plant Disease Research*, 5(1): 25-33.

ISTA (Internation Seed Testing Association) (1966). International rules of seed testing. Proc. Int. Seed Test. Ass. 31: 1-15.

Kaminski (1982). Description and culture of chickpea. Extension Bulletin 1112 Cooperative Extension Service Washington State University. Pullman, Washington.

Karunakar, R.I.; Kunwar, I.K.; Satyaprasad, K.; Ramarao, P. (1992). Histopathology of sorghum seedling roots infected by *Macrophomina phaseolina*. *Phytophylactica*, 24(4): 325-327.

Liang, J.X.; Bai, J.K. Studies on fungi causing root rot. *Indian Phytopath*. 19(1): 27-34.

Maesen, L.J.G. (1972). *Cicer:* a monograph on the genus with special reference to the chickpea (*Cicer arietinum*), its ecology and cultivation. Wageningen, The Netherlands.

Magalhaes, A.A. de, Choudhury, M.M.; Millar, A.A. Albuquerque M.M. de (1982). Effect of soil water deficit on *Macrophomina phaseolina* infection on bean Pesquisa Agropecuaria Brasileira, 17(3): 407-411.

Ahmad, M. and Mehdi, S.S.; Ahmad, M. (1992). Oil content and fatty acids of exotic sunflower inbred lines as influenced by different charcoal rot isolates. Proceedings of the 13th International Sunflower Conference Volume 2, Pisa, Italy, 7-11 September 1992, 946-956.

Meyer, W.A.; Sinclair, J.B.and Khare, M.N. (1974). Factors affecting charcoal rot of soy bean seedlings. *Phytopathology*, 64(6): 845-849.

Mishra, C.; Som, S.and Singh, B. (1988). Interaction of *Meloidogyne incognita* and *Rhizoctonia bataticola* in white jute (*Corchorus capsularis*). *Indian Journal of Agricultural Sciences*, 58(3): 234-235.

Monga, D.; Sheo Raj and Raj, S. (1994). Cultural and pathogenic variations in the isolates of *Rhizoctonia* species causing root rot of cotton. *Indian Phytopathology*, 47(4): 403-407.

Ohr, H.D.; Sims, J.J.; Grech, N.M.and Backer. Chemical treatment by Tilt in vitro. P.D.R. 80: 731-735.

Oyekan, P.O. and Naik, D.M. (1987). Fungal and bacterial diseases of soy bean in the tropics. In: Singh, S.R.; Rachie, K.O.; Dashiell, K.E. eds. Soy beans for the Tropics: Research, Production and Utilization. John Wiley & Sons Ltd, 47-52.

Patel, K.K. and Patel, A.J. (1990). Control of charcoal-rot of sesamum. *Indian Journal of Mycology and Plant Pathology*, 20(1): 62-63; 3 ref.

Pineda, J.; Nass, H. and Rodriguez, H. (1985). Effect of the inoculum density of *Macrophomina phaseoli* on infection of sesame seedlings. *Agronomia Tropical*, 35(4-6): 133-138.

Saxena, M.C. and Singh, R.B. (1986). The chickpea, ICARDA, Aleppo, Syria.

Sharma, J.K.; Singh, I. and Chhabra, H.K. (1980). Observations on the influence of *Meloidogyne incognita* and *Rhizoctonia bataticola* on okra. *Indian Journal of Nematology*, 10(2): 148-151.

Sheikh, A.H. and Ghaffar, A. (1979). Relation of sclerotial inoculum density and soil moisture to infection of field crops of *Macrophomina phaseolina*. *Pakistan Journal of Botany*, 11(2): 185-189.

Short, G.E. and Wyllie, T.D.; Ammon, V.D. (1978). Quantitative enumeration of *Macrophomina phaseolina* in soy bean tissues. *Phytopathology*, 68(5): 736-741.

Short, G.E.; Wyllie, T.D. and Bristow, P.R. (1980). Survival of *Macrophomina phaseolina* in soil and in residue of soy bean. *Phytopathology*, 70: 13-17.

Siddaramaiah, A.L.and Hegde, R.K. (1982). Influence of soil moisture on seedling infection of safflower due to *Rhizoctonia bataticola*. Current Research, University of Agricultural Sciences, Bangalore, 11(5/6): 64-65.

Simmonds, N.W., editor. (1976). Evolution of crop plants. London, New York: Longmans, 1976

Simosa, C.N.C. and Delgado, M. (1991). Virulence of four isolates of *Macrophomina phaseolina* on four sesame (*Sesamum indicum*) cultivars. *Fitopatologia Venezolana*, 4(1): 20-23.

Singh, R.D.N. and Kaiser, S.A.K.M. (1992). Role of agroclimatic factors on the incidence of charcoal rot of maize. *Advances in Plant Sciences*, 5(2): 559-568.

Singh, S.K. and Nene, Y.L. (1990). Cross inoculation studies on *Rhizoctonia bataticola* isolates from different crops. *Indian Phytopathology*, 43(3): 446-448.

Singh, S.N.; Srivastava, S.K.; Bhargava, P.K.; Khare, M.N. (1990). Chemical control of seedling mortality in cv. J.S. 72-44 and bacterial pustule in cv. Punjab-1 of soy bean (*Glycine max* (L.) Merrill). *Legume Research*, 13(1): 17-20, 16 ref.

Sobti, A.K. and Bansal, R.K. (1988). Cultural variability among three isolates of *Rhizoctonia bataticola* from groundnut. *Indian Phytopathology*, 41(1): 149-151.

Soheib, A.Y. (1983). Detection of *Fusarium moniliforme* (Sheldon) in okra seed (*Hibiscus esculentus*) its significance and control. M.Sc. Thesis, Univ. of Khartoum.

Srivastava, S.K. and Dhawan, S. (1979). Epidemiology of *Macrophomina* stem and root rot of *Brassica juncea* (L.) Czern Coss. in Northern India. Proceedings of the Indian National Science Academy, B, 45(6): 617-622.

Thanasoupoulas, C.C. (1979). Control of *Macrophomina phaseolina* by Tilt fungicide P.D.R. 54.

Todd, T.C.; Pearson, C.A.S.; Schwenk, F.W. (1987). Effect of *Heterodera glycines* on charcoal rot severity in soy bean cultivars resistant susceptible to soy bean cyst nematode. *Annals of Applied Nematology*, 1: 35-40, 29 ref.

Tosi, L. and Zazzerini, A. (1990). Influence of environmental factors and cultural techniques on sclerotium bataticola Taub. on sunflower. *Informatore Fitopatologico*, 40(2): 73-76.

Vidhyasekaran, P. and Arjunan, G. (1978). Studies on leaf blight of urd bean caused by *Macrophomina phaseoli*. *Indian Phytopathology*, 31(3): 361-362.

Yang, S.M.; Rogers, C.E. and Luciani, N.D. (1983). Transmission of *Macrophomina phaseolina* in sunflower by *Cylindrocopturus adspersus*. *Phytopathology*, 73(10): 1487-1469.

Appendix I
Culture Media

1. PDA	**Potato dextrose agar contain**
Potato extract	250 gm
Dextrose	20 gm
Agar	15 gm
Distilled water	1000 ml

2. Czapeks Dox Agar

Dipotasium phosphate	1.0 gm
Sodium nitrate	0.5 gm
Magnesium sulphate	0.5 gm
Potassium chloride	0.5 gm
Ferrous sulphate	0.1 gm
Sucrose	20.0 gm
Distilled water	1000 ml

3. Malt Extract Agar (MEA)

Malt extract	30 gm
Agar	20 gm
Distilled water	1000 ml

Appendix 2

Fungicides

1. Bayleton: (Trade name)

Common name: Triadimefon

Chemical name: 1-4 (chlorophenoxy-3,3-dimethyl-9-(1-H-1,2,4-riazole-1-yl) Butan-zone

Mode of action: Systemic fungicide with protective, curative and eradicant action, absorbed by the roots and leaves with readily translocation in young growing tissues.

2. Tilt: (Trade name)

Chemical name: 1-2-3 dichlorophenyl-4-propyl-3-1 dioclson-2-xd methyl-1-2-4 triazol.

Mode of action: Systemic fungicide used for inhibiting the first stage of the fungal growth.

ملخص الأطروحة

أجري هذا البحث على أربعة أصناف من بذور الحمص لمعرفة الفطريات المحمولة على أو داخل هذه البذور وهذه الأصناف صنف من زالنجي وصنف من جبل مرة وصنفان من الحديبة (شندي وود حامد).

أجريت الاختبارات الموصى بها لفحص البذور (ISTA, 1966) وهي اختبار ورق الترشيح المبلل واختبار طبق الآجار إضافة إلى الفحص الجاف للبذور.

الفحص الجاف للبذور أوضح أن هذه الأصناف تحتوي على بذور ملونة وأخرى مشوهة وأخرى مكسورة وبعد معاملة البذور بهيدروكلوريد الصوديوم 1% (Sodium hypochloride)أظهرت التجارب وجود الفطريات التالية .*Alternara* sp و*Drechslera* sp. و*Rhizctonia bataticola* وكانت نسبة الفطر *Rhizctonia bataticola* عالية عزل الفطر وأجريت عليه عدة اختبارات أوضحت أن Malt extract هي البيئة المثلى لنمو الفطر.

وعندما تمت معاملة الفطر بمبيد البايلتون أدى إلى الحد من نمو الفطر حتى في التركيز الأدنى 10 ppm بينمات مبيد التلت أدى إلى توقف نمو الفطر تماماً.

بسم الله الرحمن الرحيم

بعض الدراسات على فطر *Macrophomina phaseolina* المعزول من بذور الحمص الجافة

تقديم

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بكالوريوس العلوم في الزراعة

جامعة السودان للعلوم والتكنولوجيا

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المشرف

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