CHABTER ONE

ENTRODUCTION

Chickpea (*Ciceri arietinum* L.) is belong to the family fabaceae and has an obscure origin it is one of the most important food legume in Sudan. In addition, it is an important cash crop providing major source of low cost protein for masses of low-income groups apart from being an important source of dietary protein for human consumption this pulse crop is also important for the management of soil fertility due to its nitrogen fixing ability (Maili 2001 kantar *et. al* 2007). Chickpea was first cultivated in an area of South Eastern Turkey and joining Syria (Toker 2009) but is now cultivated throughout the semi-arid region of the world (Jordan and rao1987) (knights *et al* 2007). The most common commercial varieties are the small dark desi type of Indian origin or the larger light colored, kabuli type from the Mediterranean and Middle East regions. Desi chickpea account for about 90% of the world's current commercial production. It an upright annual herbaceous legume form 30-70cm.The total tall that is well adapted to arid climates. The compound leaves composed of 10 – 16 leaflets with serrated edges are covered with fine hairs that limit water loss from evapotranspiration; some of the hairs secrete an acid that helps protect the plant against attack by insects. The leaflets can also fold up slightly during hot dry weather further limiting water loss a large deep tap root allows the plant access to deeper water supplies the plant produces small pea-like flowers generally white in the kabuli type violet in the desi type the purple coloring is caused by a chemical called Anthocyanin. Because of the anthocyanin, the purple – flowered varieties also have darker leaves stems and seed coatings flowers later pods.

Chickpea is valued for its nutritive seeds with high protein content 25.3-28.9%. Also very high in clattery fiber and hence healthy source of carbohydrates for person with insulin sensitively or diabetes .chickpea are low in fat and most of this is polyunsaturated. Chickpea is a cool – season annual that requires too or so days to reach harvest. Chickpea as are frost to learnt but grow best where day time temperatures range between 70and80 and where night time temperatures do not hip dip blew 65f. Legume grains are the most important type of food grains after cereals in many part of the world. The major constraints facing the productivity of chickpea are the losses caused by diseases insets nematodes and parasitic weeds the main fungal pathogens that attack chickpea are *Fusarium* oxysporum - Cicero's causing by Ascochyla *rabies*. Fusarium wilt and root – rot are the most important diseases of food legumes in the Sudan (Ali ,1989) .The disease is very important in the Sudan especially in areas where farmers do not adhere to crop rotations the disease incidence was monitored at Hudeiba and Rubatab locations (north Sudan) . The occurrence of will and root rot disease Complex has been observed in recent years in Khartoum area with high levels of severity (Hussein, 2002). Traditionally ,plant fungal disease are controlled by synthetic fungicide, which increase agriculture costs and contaminate the environment with very toxic substance (Carvallo ,2004) A possible alternative to solve such problems is the use of plant able to produce antifungal substances (Miranda , 2003) .

The thesis is therefore intended to:

I. Isolate and identify the causal agents of wilt disease of chickpea in the main producing area selected in Sudan.

II. Investigate the efficacy of alternative control measures involving Argel (*Solenostemma argel*) against *Fusarium oxysporum* f.sp. Ciceris.

CHAPTER TOW

LITERATUR REVIEW

2.1 **Chickpea**

Chickpea is considred as one the most important food pulse legumes providing a major source of low-cost protein for masses of low – income groups.

2.1.1 Scientific Classification

Kingdom: Plantae

Division; Magnoliophyta

Class: Magnoliopsida

Order : Fabaies

Family: Fabaceae

Sub family: Faboideae

Genus: *Cicer*

Species : *Ciceri arietinum*

Ciceri arietinum L belongs to the Dicotyledonous crops, which are the most important family with an annual world market value. Furthermore, .chickpea was second in importance to cereals among world food production [Winter *et al*. 2003].

2.1.2 Types

There are two main kinds of chickpea:“ Desi “ which has small darker seeds and rough coat cultivated mostly in the India subcontinent Ethiopia Mexico and Iran Desi chickpea account for about 90% of the worlds current commercial production

Kabuli Which has lighter color red larger seeds and smoother coat mainly grew in southern Europe northern Africa Afghanistan Pakistan and Chile also introduced during the 18 the century to the Indian subcontinent Mansfield world database of agricultural and horticultural crops *Ciceri arietinum*.

2.1.3 Botanical features

Chickpea is upright annual herbaceous legume from 30-70 cm tall is well adapted to arid climates .The compound leaves composed of 10-60 leaflets with serrated edges are covered with fine hairs that limit water loss from evapotranspiration .Some of the hairs that protect the plant against attack by insects . The leaflets can also fold up slightly during hot dry weather further limiting water loss and a large deep taproot allows the plant access to deeper water supplies.

The plant produces small pea-like flours generally white in the kabuli type and violet in the desi type the purple coloring is caused by a chemical called anthocyanin .Because of the anthocyanin the purple-flowered varieties also have darker leaves stems and seed coatings .Flowers and later pods from axillaries nodes and each pod contains form two to at three seeds.

The root system of the chickpea also secretes an acid that helps the plant take up phosphorus from the soil allowing growth on soils with limited supplies of this essential element.

2.1.4 Uses:

Chickpea is consumed as a dry pulse crop or as a green vegetable, with the former use being most common seeds average about 20% protein 5% fat and 55% carbohydrate seeds are sold in markets either dry or canned. Common uses in United States are in soups vegetable combinations or as a component of fresh salads in restaurant salad, some livestock feeding trials have been conducted and these show chickpea to be a good source of protein for feeds except that the amino acids methionine and cysteine are deficient.

Growth habits, plants multiple branched spreading growth habit annuals ranging from 8 to 40 in tall. Some chickpea varieties have compound leaves (8 to 20 leaflets) and some have simple leaves which are pubescent (hairy) in appearance chickpea leaves exude malic and oxalic acids. Kabuli (large seeded =800seed/IB) varieties are generally teller than the desi (small –seeded varieties).

Because of its deep tap, root system chickpea can withstand drought conditions by extracting water from deeper in the soil profile.

Flowers (self – pollinated) which are borne in groups of two or three are ½ to 1 in. Each flower produces a short pubescent pod, which is 3/4 to 2in long and with appears to be inflated. One or two seeds (1/2 to 1 in .diameter) are present in each pod the seeds come with either rough or smooth surface and can be crème yellow brown black or green in color, There is a definite groove visible between the cotyledons about two –thirds of the way around the seed with a beak –like structure present.

2.1.5Environment Requirement:

1.2.5.1 Climate

Chickpea is a cool season annual crop performing optimally in 70 to 80 f day time temperatures and 64 to 70 f night temperatures. They produce good yields in drier conditions because of their deep taproot. Heavier rainfall seasons (over 30 in annually) show reduced yields due to disease outbreaks and stem lodging problems from the excessive vegetative growth .Areas with lighter, well distributed rainfall patterns have produced the highest yield and quality chickpea seed .

2.1.5.2 Soil:

Chickpea dose best of fertile sandy loam soils with good internal drainage .Good drainage necessary because even short periods of flooded or waterlogged fields reduce growth and increases susceptibility to root and stem rots.

2.1.5.3 Weed Control:

2.1.5.3.1 Mechanical:

Chickpea is not very competitive with weeds so they should be planted only on fields that have few if any major weed problems especially perennial weeds such as quack grass and Canada thistle.

Rotary hoeing and /or field cultivating in wider row spacing should be used as necessary to control weed populations in chickpea. Early weed competition is more damaging to yield than later emerging weeds. Avoid extensive damage to plants and cultivate when leaves and stems are dry to reduce spread of disease organisms.

2.1.5.3.1.2Chemical:

The herbicide method bachelor (dual) can be applied as a replant incorporated or reemergence treatment .It gives excellent annual grass fair to good annual broadleaf control .A rotary hoe could be used in chickpea in the same manner as with soybean. Row cultivation is not practical due to the narrow row spacing.

If annual grasses or quack grass are abundant after the crop emerges a post emergence application of Sethoxydim (post) should be considered. Treated when the grasses are 4 to 6 in. Tall .a 1pt/ acre rate controls most annual grasses; check the label and select the rate appropriate for your weed species. Always use 1qt/acre of Dash or a crop oil concentrate when post is applied. Chickpea has been grouped on some herbicide label with other dry pod harvested crops such as field dean or chickpea has been or adzuki bean. This could allow use of herbicide cleared in those crops to be used on chickpea. Read labels carefully and seek clarification from the company involved before using any herbicide on your chickpea crop. Be sure to ask a company representative your extension agent or crop consultant for the most recent information and follow the label directions exactly .Because chickpea is a lesser-grown crop in Wisconsin and Minnesota label clearance from other states may not apply.

2.1.5.3.1.4 Diseases and their control:

Ascochyla blight, Rhizoctonia root rot, pythium rot, Fusarium wilt, white mold bacterial blight and certain viruses are possible disease problems in production fields of chickpea. These are typical diseases, which affect other pulse or legume crops, and they are stressed by periods of high rainfall high humidity and high temperatures.

These are best controlled by using good quality seed proper crop rotation proper tillage practices to bury diseased and disease resistant varieties if available.

Contact your extension agent or crop consultant for identification of disease organism's threshold value determinations and control or management suggestions

2.1.5.5 Harvesting

Chickpea can be harvested direct swathed prior to combining depending upon uniformity of maturity and weed problems. About one week of good drying weather is required in the swath.

Chickpea can be swathed when the plants are yellowing and the pods are their mature color .This should be done when the plans are slightly damp to facilitate forming the swath without yield loss .When the vines pods and seeds in the windrow are dry enough [seed moisture about 13%]the swath con be combined. Seed color is important (buyers prefer a yellowish – crème color) so greenish and brown seeds are generally an acceptable .slight bleaching does occur in the swash about 1% in mature color seed is allowed before deductions are implemented.

Adjust the combine screen size cylinder speed concave clearance and air flow carefully to maintain a quality seed with little damage or excessive trash .

2.1.5.6 Drying and storage:

Moisture content should be around 10 to 12 % to prevent insect and or disease out breaks in storage. Because of their relatively large seed, size chickpea con be dried slightly wit ambient temperature air flow through thin layers in a regular storage bin.

Storage system should be carefully fumigated before storing chickpea and all storage areas should be monitored regularly to identify to potential problems early.

2.2 *Fusarium oxysporum*

F. oxysporum is a seed and soil –borne fungi pathogen that causes Fusarium wilt of chickpea (Haware .1990; Neneand Reddy.1987).

2.2.1 Classification:

Kingdom: fungi

Phylum: Ascomycota

Class: Sordariomycetes;

Sub Classes: Hypocreonycetidae

Order: Hypocreales

Family: Nectriaceae

Genus: Fusarium

Species: *Fusarium oxysporum f .sp.* Ciceris.

2.2.2 Description

Fusarium oxysporum is a common soil inhabitant and produces three types of asexual spores; macro conidia micro conidia and chlamgdospores .The macro conidia are straight to slightly curved slender thin walled usually with three or four septa a foot –shaped basal cell and a tapered and curved apical cell.

They are generally produced from phialides on conidiophores by basipetal division.

They are important in secondary infection .

The micro conidia are ellipsoidal and either have no septum or a single one. They are formed from phialides in false heads by basipetal division. They are important in secondary infection .The Chlamgdospores have thick walls .They are formed from hyphae or alternatively by the modification of hyphae cells .they are import ant as endurance organs in soils where they act as inoculate in primary infection. The teleomorph or sexual reproductive stage of *F.oxysporum* is unknown.

2.2.3Distribution

Overall, the distribution of *Fusarium* oxysporum is known to be cosmopolitan. However, the different special forms (f.sp) of *F.oxysporum* often have varying degrees of distribution.

2.2.4 Symptoms

The fungus enters the vascular system of the infected plant via the roots it produces enzymes that degrade the cell walls so that gels are formed that block the plants transport system. Discoloration of the internal tissues progresses from the roots to the aerial parts of the plant yellowing, wilting of the foliage occur, and finally there is necrosis.

It is possible to identify affected seedlings approximately three weeks after sowing as they display preliminary symptoms such as drooping and pale- colored leaves. Later they collapse to a prostrate position and will be found to have shrunken stems both above and below ground level. When adult plants are affected, they exhibit wilting symptoms that progress from the petioles and younger leaves in two or three days to the whole plant. The later stage of the disease all leaves turn

yellow discoloration of the pith and xylem occurs in the roots and can be seen when they are cut longitudinally.

2.2.5 Disease Cycle

F. oxysporum is an abundant and active saprophyte in soil and organic matter with some specific forms that are plant pathogenic (Smith et al 1988). Its saprophytic ability enables it to survive in the soil between crop cycles in infected plant debris. The fungus can survive either as mycelium or as any of its three different spore types (Agrios 1988).

Healthy plants can become infected by *f. oxysporum* if soil in which they are growing is contaminated with the fungus. The fungus can invade a plant with its either sporangial germ tube or mycelium by invading the plants roots. The roots can be infected directly through the root tips (Agrios 1988). The mycelium grows through the root cortex in tercellulary. When the mycelium reaches the xylem it, invades the vessels through the xylems pits. At this point, the mycelium remains in the vessels where it usually advances upwards toward the stem and crown of the plant. As it grows, the mycelium drenches and produces micro conidia, which are carried up ward within the vessel by way of the plants sap stream. When the micro conidia germinate, the mycelium can penetrate the upper wall of the xylem vessel enabling more micro conidia to be produced in the next vessel. the fungus can also advance laterally as the mycelium penetrates the adjacent xylem vessels through the xylem pits (Agrios 1988)

Due to the growth of the fungus within the plants vascular tissue the plants water supply is greatly affected. This lack of water induces the leaves stomata to close the leaves wilt and the plant eventually dies. It is at this point that the fungus in vades the plants parenchymatous tissue until it finally reaches the surface of the

dead tissue where it sporulates abundantly (Agrios 1988) the resulting spores can then be used as new inoculum for fur the r spread the fungus.

2.2.6 Epidemiology

F.oxysporum is primarily spread over short distances by irrigation water and contaminated farm equipment. The fungus can also be spread over long distances either in infected transplants or in soil. Although the fungus can sometimes infect the fruit and contaminate its seed the spread of the fungus by way of the seed is very rare (Agrios 1988) It is also possible that the spread by wind.

2.2.7 Management

Management of Fusarium wilt chickpea would be best achieved if those disease control measures are used within an integrated management strategy where by use were combined either simultaneously or in a sequence (Haware *et al*., 1990).

2.2.1 Culture control

The culture control is the only practical measure for controlling the diseases in the field .The wilt fungus is widespread and so persistent in soils that seed bed sterilization and crop rotation although always sound practices but are of limited value .Soil sterilization is too expensive for greenhouse grown chickpea plant (Agrios.2005).

Moreover, use of healthy seed and ransplants is of course mandatory and hot water treatment of seed suspected of being infected should precede planting (Agrios. 2005).

As mentioned above Fusarium wilts affect and cause severe losses on most vegetable and flowers several field crops as cotton, tobacco, banana, plantain,

coffee ,sugarcane and a few shade trees .Fusarium wilts are most severe under warm soil conditions and green house (Agrios.2005).

Prevent spreading of the pathogen to disease free areas by using clean tools and equipment (Agrios. 1997; Jone *et at* .1997).

2.2.2 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 (Ageafotis *et al*., 2002). However, a number of researchers studied the step of validation of traditional uses of antimicrobial compounds in higher plants. Accordingly, the effect of different plants extracts on the germination and growth of many fungal pathogens have been reported (Agrafotis *et al*., 2002).

They used many strategies for controlling Fusarium wilt such as plant extracts, cultural practices and other methods. However, they are control of fungal disease in plant are considered as an interesting alternative to synthetic fungicides due to plant essential oils have been tested against *F.oxysporum* species for inhibitor effect and control efficacy under greenhouse condition (Bowers, and Locke,2000). If natural plant products can reduce populations of soil borne pathogens and control diseases development, than these plant extracts have potential as environmentally safe alternatives and as component in integrated pest management programs. Chand and Singh (2005 reported that the plant extracts, VIZ *Calotropis procera*, *Eucalyptus globules*, *Jatropha crocus.*

2.2.3 Biology Control:

In solid media culture such as potato dextrose agar (PDA) the different special forms of *F.oxysporum* can have varying appearances .in general the aerial mycelium first violet to dark purple – according to the strain (or special form) of *F. oxysporum* if sporodochia are abundant the culture may appear cream or orange in color (Smith *et al* 1988).

F.oxysporum produces three types of asexual spores:" micro conidia, macro conidia and Chlamydo spores (Agrios 1988]). Micro conidia are one or two celled and are the type of spore most abundantly and frequently produced by the fungus under all conditions .It is also the type of spore most frequently produced within the vessels of infected plants (macro conidia) are three to five celled gradually pointed and curved toward the ends. The spores are commonly found on the surface of plants killed by this pathogen as well as in sporodochia like groups (chlamydo spores) are round thick-walled spores produced either terminally or intercalary on older mycelium or in macro conidia . These spores are either one or two celled (Agrios 1988).

2.2.4 Chemical control

Presently, Anon (1994) and Ristaino *et al*., (1997) reported that methyl bromide fumigation is used extensively for chickpea production in some geographical areas in addition to reducing or elimination soil borne disease like Fusarium wilt. Fumigation allows more rapid transplant growth allowing for earlier harvesting and optimizes fresh markets .The use of methyl promide may be curtailed in near future and alternative chemicals are being examined.

CHAPTER THREE

MATERIALS AND METHODS

3.1.1 Isolation of the pathogens

Infect chickpea roots showing symptoms of the disease were obtained from Shambt research station (sick block). The infected roots were kept paper bags. parts showing disease symptoms were cut into small sections 0.5-1cm washed thoroughly with tap water and surface sterilized by immersing them in diluted 1:4 Clorox (NaOCL) for 5minutes rinsed three time in changes of sterilized distilled water to remove the adhering Clorox and dried on sterilized filter papers. Then ready for culturing. A culture medium potato Dextrose Agar (PDA) was used the medium was supplemented with anti-biotic (0.05g/1)as bacteriostatic agent (Anon 1981) the medium was poured in 9 cm petri dishes .Five sections of the dried parts were aseptically placed in a petri dish and incubated at 28c.subcultures were later prepared to get pure cultures. Slides were prepared from these pure cultures and examined microscopically (x: 40).also the fungus isolated from infested soil.

3.2 Identification of the pathogens

The identification of the fungi was based on visual culture characteristics mainly the growth patterns and pigmentation. Further microscopic examinations were carried out for mycelia and conidia structure based using (Booths key 1977).

3.3 Growth rate of the pathogen

Pure cultures of the fungi *Fusarium* oxysporum f.sp Ciceri was prepared using mycelia from the edge of 7 days –old cultures of the fungi on PDA which were transferred to the center of other petri dishes aseptically and incubated at 28c . The

linear growth was first measured after 48hrs then regularly at intervals of 24hrs. The linear growth was assessed in cm.

3.3 Effects of botanicals:

The aim of this experiment was to study the antifungal activities of plant extracts on the growth of *F. oxysporum in vivo*. Extracts from plants were tested for their effects on the fungus. The plants used was Argel (*Solenostemma argel*)**.**

3.3.1 Sample Preparation

3.3.2 Collection of plant samples

Argel was obtained from the Department of Botany, Faculty of Agriculture, and University of Khartoum. The plants or the plant part were washed with tap water, air-dried under shade, and each part, packed in a separate envelope, dried in an oven at 50°C for 48 h. A home crusher into coarse powder crushed the dried plants.

Sterilized earthen pots (25 cm diameter) were filled with autoclaved potting mixture of soil : sand (1:1) and as per treatment details the potting mixture was amended with powders of Argel.

The pots were subsequently, inoculated with the fungus (*F. oxysporum* f. sp. *Ciceri* 100 g/kg soil). Seeds of chickpea were sown (five seeds/pot). Treatment was arranged in complete Randomized design (CRD) with four replicates. The pots were lightly irrigated and incubated at room temperature for one week

Potting mixture inoculated with test fungus sown to untreated seeds was included as control. Observations on percent seed germination; pre/pot, emergence and wilting were recorded periodically up to harvest.

2.5 Statistical analysis procedure

Data generated was subjected to complete Randomized design (CRD)

Design analysis

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