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Fusarium Wilt Disease of Chickpea in Sudan: Incidence, Pathogen Genetic Variability and Screening for Resistance

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Dedication

To my lovely Family,

my husband&mykids

Omeyma

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CHAPTER THREE

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List of abbreviations

Foc	*Fusarium oxysporum* f. sp. *ciceris*
ha	Hectare
g	Gram
Kg	Kilogram
PDA	Potato Dextrose Agar media
DNA	Deoxyribonucleic Acid
RAPD	Random Amplified Polymorphic DNA
SSM	Sorghum Sand Mixture
SSR	Simple Sequence Repeats primers
SCAR	Sequence Characterized Amplified Region primers
PCR	Polymerase Chain Reaction
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
W/W	Weight/Weight

ABSTRACT

Chickpea (*Cice rarietinum* L) is a cool season food legume and an important cash crop in the Sudan. The crop was traditionally grown on residual moisture of the flood in northern Sudan and recently it is introduced to the central parts of the country as an irrigated crop. Fusarium wilt disease caused by the fungus *Fusarium oxysporum*. f. sp. *ciceris* (*Foc*) is one of the most important vascular wilt diseases of the crop in Sudan, which affects its production. A survey was conducted in seasons 2010/2011 and 2011/2012 in different chickpea production areas to assess the incidence of the disease, its correlation to farmers' cultural practices and to identify the genetic diversity of the pathogen population in Sudan. In this regard, 170 samples of chickpea wilted plants were collected from six States in the country (Northern State, River Nile State, Khartoum State, Gezira State, Sennar State and Kassala State). Information about the cultural practices done by farmers during the growing seasons, GPS data and samples of chickpea wilted plants were collected in each location. The survey data were analyzed using SPSS 16.0 Program and a map for the disease was madeusing GPS data. The pathogen was isolated from collected samples and purified in single spore cultures in vitro and subjected to cultural, morphological and pathogenicity studies. To identify the genetic diversity among the Sudanese *Foc* isolates, 90 distinct isolates were studied including 76 isolates obtained from different chickpea growing regions of Sudan and 14 isolates obtained from Syria and Lebanon for comparison. The DNA of all the isolates were extracted and subjected to molecular characterization by three types of molecular markers. The PCR analysis were carried out using four Random Amplified Polymorphic DNA (RAPD) primers, three Simple Sequence Repeats (SSR) primer pairs, 5 Sequence Characterized Amplified Region primers (SCAR) and 2 specific *Foc* identification primer sets. Gezira State gained the highest chickpea surveyed area and the most popular chickpea

variety grown was the small kabuli "Baladi" variety. This variety occupied about 64% of the total surveyed area and was the most susceptible variety to the disease similar to Shendi and Jebel Marra cultivars (20-25% disease incidence). The incidence of the disease in the heavy clay soils of central and southern Gezira State (21 and 27%) was higher than that in the light clay and sandy clay soils (11-12%). The area under chickpea preceded by cereals in the rotation accounted for 40% of the total surveyed area followed by fallow (15%) and less than 10% for vegetables, and monocropping. Disease incidence was the highest in the monocropping system (42%), whereas in chickpea fields preceded by cereals, cotton, fallow and vegetables, it was 22, 20, 12 and 8%, respectively. Sowing date predominantly used by farmers was mid November. Early sowings during this period were subjected to high wilt disease incidence (31%) as compared to late December sowings that exhibited only 5% disease incidence.The seeding rate varied from farmer to farmer and the disease reached 50% in fields grown with more than 80 kg/ha. More than 40% of the farmers watered their crops at 2 weeks interval. The crop subjected to shorter (weekly) watering intervals had lower disease incidence (16%) than that subjected to longer intervals (22 and 24%). Around 54% of the farmers did not apply any kind of fertilizers to chickpea and 45% apply starter dose of Urea (2N/ha) and very few farmers added foliar fertilizers. Addition of fertilizers had no significant effect on disease incidence. Testing of 25 *Foc* isolates collected from Gezira, Khartoum, Sennar, Kassala and River Nile States with a set of chickpea differential varieties resulted in resistant reaction of these differentials to the tested isolates. This reaction is similar to the reaction of these differentials to race 0 in previous studies. When the isolates were cultured on PDA media, distinct variations were noted among the isolates with respect to colony diameter, texture and colony color, measurements of macro and micro conidia, abundance and absence of chlamydospores. The size of microconidia was in a range of 3.75-12.9 x 2.1- 3.4 µm. The longest and the shortest microconidia

were observed in isolates of central and southern Gezira State, respectively. There were no significant variations among micrconidia length and width of all the isolates. Generally, macroconidia size of all isolates was in a range of 17.5-42.5 x 2.5-6.25 μm and the longest macroconidia was observed in Altalha isolate of central Gezira, whereas the shortest was observed in Alburgaig (Northern State) and Hudeiba (River Nile State) isolates. The widest macroconidia was observed in Daressalm area of central Gezira. Chlamydospores were detected only in isolates from central Gezira, Sennar, Hudeiba and Shambat sick plots and Syrian isolates. Four RAPD and three SSR primers were used to assess genetic diversity among 90 isolates collected from six chickpea growing states in Sudan. They were compared to Syrian and Lebanese isolates with known race identity. It is apparent that all the isolates are *Fusarium oxysporum* f. sp. *ciceris* but they are different from the Syrian and Lebanese isolates. Based on the coefficient of similarity, the isolates were grouped into two different major clusters and seven sub clusters in the dendrogram. The minimum dissimilarity value between the isolates was 0.1 and the maximum value was 1. These clusters differentiated the *Foc* isolates of Sudan based on the races nomenclature to race 0, 2 and unidentified race. The cluster analysis clearly distinguished the unidentified Foc strains obtained from central Gezira State from the other *Foc* isolates. Race 0 is widely distributed in central Sudan, while the unidentified race is restricted to Gezira State. Race 2 is distributed in Northern State, River Nile State and northern part of Gezira State. The Syrian and Lebanese *Foc* isolates which have been included for comparison were sub clustered separately which coincided with their expected races 1B/C and 6, respectively. Twenty chickpea germplasm were screened against the three identified races. The cultivar Hawata showed resistant reaction to the three tested *Foc* races, while Shendi and Jebel Marra cultivars showed susceptible reaction to race 0 and highly susceptible reaction to the other two races. The other genotypes showed susceptible reactions to the unidentified race and

variable reactions to races 2 and 0.It is evident from this study thatthe specific molecular markers used are the most rapid, reliableand effective tools in characterization and race identification of *Fusarium oxysporum* f. sp. *ciceris*. In addition, these findings will contribute to not only design and develop effective management strategies for chickpea wilt disease but will also help the breeders to design effective disease resistance breeding programs in chickpea.

مرض الذبول الفيوزارمي في محصول الحمص بالسودان : نسبة حدوث المرض, التباين الجيني, للكائن الممرض وتقييم المقاومة

ملخص الدراسة

الحمص أو الكبكبي (*Cicer arietinum* L) هو احد أهم المحاصيل البقولية الغذائية والنقدية التي تزرع في موسم الشتاء في السودان. يزرع المحصول تقليديا في شمال السودان الي ان انتقلت زراعته حديثا الي المناطق الوسطي من البلاد كمحصول مروي. مرض الذبول الفيوزاريومي بمحصول الحمص والذي يسببه الفطر *Fusarium oxysporum* f. sp. *ciceris* واحد من اهم أمراض الذبول الوعائي بالمحصول في السودان و يؤدي الي تدني كبير في الانتاجية. تم إجراء مسح لهذا المرض لدراسة التوزيع الجغرافي للمرض ومن ثم الكائن المسبب, دراسة علاقة العمليات الفلاحية بانتشار المرض ثم دراسة التنوع الوراثي للفطر المسبب للمرضفي مناطق زراعة المحصول. تم جمع 170 عينة من نباتات الحمص المصابة من ست ولايات بالسودان هي الشمالية ، نهر النيل (شمال السودان)، الخرطوم، الجزيرة, سنار وكسلا (وسط وشرق السودان). تم تسجيل المعلومات عن العمليات الفلاحية التي تمت خلال موسم زراعة المحصول وتم تسجيل بيانات خطوط الطول والعرض والارتفاع في كل موقع بجهاز تحديد المواقع الجغرافيه (GPS) ثم جمعت عينات مصابه ظهرت عليها اعراض مرض الزبول الفيوزارمي. تم عزل وتنقية العينات من جرثومة واحدة في المختبرثم اخضعت الي دراسة عامة لمعرفة لون وملمس وحجم نمو الفطروطول وعرض جراثيم الفطر تحت ظروف المعمل. لمعرفة التنوع الجيني للسلالات السودانية للفطر (*Foc*)، تمت دراسة 90عزلة من الفيوزاريوم (76 عزلة من السودان و 14 عزلة من سوريا ولبنان للمقارنة) تم عزل الفطر من جميع العينات المصابة وتنقيتها في المختبر ثم تم استخلاص الحمض النووي لجميع العزلات وأجري عليها تحليل (PCR) باستخدام أربعة أنواع من الواسمات الجزيئية 4) (RAPDs, 3 SSRs, 8 SCARs, 5 specific for Foc. كان صنف الحمص البلدي الكابولي الأكثر انتشارا بين المزارعين (64% من جملة المساحة المزروعة) والأكثرعرضة لمرض الذبول مع صنفي شندي وجبل مره بنسبة اصابة 20-25%. محصول الحمص المزروع في التربة لطينية الثقيلة في ولاية الجزيرة كان الأكثر اصابة بالمرض (21-27% اصابة)، في حين تراوحت نسبة المرض في المحصول المزروع في الأراضي الطينية الخفيفة والرملية بين 11-12%. من أهم المحاصيل التي سبقت الحمص في الدورة الزراعية الغلال بنسبة بلغت 40%ثم البور والحمص والخضروات. في حال الزراعة المتتابعة بالحمص كانت نسبة المرض هي الأعلي 42% ثم الغلال, القطن, البور والخضروات بنسبة 22, 20, 12 و 10%, علي التوالي. تاريخ الزراعة الأكثر شيوعاً بين المزارعين هو منتصف نوفمبر (تشرين الثاني) حيث كانت الزراعات المبكرة خلال هذه الفترة الأكثر عرضة لمرض الذبول(31%), اما الزراعة المتأخرة للمحصول في أواخر نوفمبر وأوائل ديسمبر (كانون الاول) كانت الأقل اصابة بالمرض(5%). معدل البذاريختلف من مزارع لآخر والمرض كان أكثر شدة في الكثافة

النباتية العالية من المحصول(50%) عندما كان معدل البذار 80 كجم للهكتار.الغالبية العظمى من المزارعين(40%) يروون محصولهم كل اسبوعين. اما فترات الري الأطول تعرض المحصول إلى نسب عالية من المرض(24%).حوالي 54٪ من المزارعين لايضيفون أي نوع من الأسمدة للمحصول و45٪ منهم يضيفون جرعة تنشيطية من اليوريا وعدد قليل منهم يرشون الأسمدة الورقية, في حين ان اضافة الأسمدة لمحصول الحمص ليس لها اثر واضح في الحد من المرض. تم عزل وتنقية عينات الفطر من العينات المصابه وتم اجراء الدراسات المورفولوجية والقدرات الامراضية لهذه العزلات ووفقا لذلك وجد من خلال هذه الدراسه ان جميع العزلات تنتمي إلي الفطر*Fusarium oxysporum* f. sp. *ciceris* وقد لوحظ وجود اختلافات واضحة بين السلالات فيما يتعلق بمعدل النمو وشكل الفطر، لون المستعمرة ، التبوغ ،عددوشكل البوغات ووجود chlamydospores. كما تمت دراسة لمعرفة القدرة الامراضيه لهذه العزلات في أصناف الحمص التفريقية (Differential varieties) فتمت عدوي مجموعة من نباتات الحمص لتعريف سلالات الفطرعن طريق خلط الفطر مع التربة ثم زراعة اصناف الحمص فيها ,لقد اظهرت جميع الاصناف التفريقية مقاومة للسلالات المستخدمة في الدراسة ونسبة للنقص في بذورالأصناف التفريقية لم تتم دراسة كل السلالات المعزولة. المدي العام لطول وعرض microcnidia بين (2.1-3.4*3.75-12.9)ميكرون. وقد لوحظت أطول وأصغر microcondia في عزلات وسط وجنوب ولاية الجزيرة. لم تكن هناك اختلافات كبيرة بين طول وعرض micrconidia في جميع العزلات. عموما كانت macroconidia في جميع العزلات بين 17.5- 42.5 X 2.5 -6.25 ميكرون ولوحظ أطول macroconidia في الطلحة بوسط الجزيرة بينما كانت الأصغر في البرقيق (الولاية الشمالية) والحديبة (ولاية نهر النيل). وقد لوحظ ان أعرض macroconidia كانت في منطقة دار السلام بوسط الجزيرة. ولقد لوحظ وجود الأبواغ الكلاميدية (Clamydospores) فقط في العزلات من وسط الجزيرة والعزلات السورية. استنادا إلى معامل التشابه الجيني , فقد انقسمت هذه العزلات الي مجموعتين رئيسيتين وسبع مجموعات فرعية في dendrogram. مجموعة تشمل العزلات السودانيه التي تم عزلها من جنوب ووسط ولاية الجزيرة و سنار وشمبات وولاية الخرطوم وتم تعريفها بالسلالة 0 (Race 0) وسلالة أخري لم يتم التعرف عليها (Race X) وتشمل العزلات من القسم الاوسط ولاية الجزيرة. المجموعة الثانيه تضم العزلات من القسم الشمالي من ولاية الجزيرة والولاية الشماليه وتم تعريفها بالسلالة 2 (Race2) وتضم هذه المجموعه ايضا العزلات السورية واللبنانية وتم تعريفهما بالسلالة 6 و1B/C,علي التوالي. السلالة 0 وجدت في نطاق واسع في وسط السودان, بينما السلالة الغير معروفة انحصرت في ولاية الجزيرة والسلالة 2 في الولاية الشمالية ونهر النيل وشمال الجزيرة. كما تمت دراسة التعرف علي مقاومة 20 من الأصول الوراثية لمحصول الحمص مع الثلاث سلالات من الفطر التي تم التعرف عليها ولقد أظهر الصنف حواتة مقاومة للثلاث سلالات بينما أظهر الصنفان شندي وجبل مرة حساسية لهذه السلالات.ولقد أظهرت باقي الاصناف درجات مختلفة من المقاومة والحساسية لهذه السلالات. هذه النتائج تشير إلى ان التحليل عن طريق التقنيات الجزيئية الحديثة للتمييز

بشكل واضح بين السلالات من فطر(*Foc*) قد تكون هي الطريقة الأمثل والأسرع في العمل البحثي لمعرفة مسببات الأمراض ودراستها جزيئيا وأن هذه الدراسة يمكن أن تكون مفيدة جدا ليس فقط لتصميم وتطوير استراتيجيات المكافحة الفاعلة لمرض الذبول الفيوزارمي في الحمص ولكن أيضا مفيدة لمربي محصول الحمص لإنتاج الأصناف المقاومة للمرض.

CHAPTER ONE

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important grain legumes in many Asian and African countries. It is one of the first grain legumes domesticated in the Old World and is thought to have originated in present day from southeastern Turkey and northern Syria. It is the world's third most important pulse crop after beans and peas and it is a major source of human and animal food especially for poor and subsistence farmers (Sivaramakrishnan *et al.*, 2002). Chickpea is also valued for its beneficial effects in improving soil fertility, particularly in dry lands, and thus significantly contributing in the sustainability and profitability of production systems (Singh and Saxena, 1996).

The major producing areas of chickpea in Sudan were Wad Hamid and Salawa in the northernSudan.Chickpea of small kabuli type was traditionally grown in northern Sudan until early1990 in an area of about 800- 2000 ha(Sheikh Mohamedand Van Rheenen, 1991).

During the past few years, chickpea growing areas extended to the central parts of the country as an irrigated crop as its production in the northern states retreated. The harvested area in Sudan increased to more than 12000 ha (FAOSTAT, 2012).

Among grain legumes, chickpea contributes to more than 50% of the cultivated area in Sudan in season 2012/2013 (Khalifa, personal communication).Compared with other food crops, especially cereals, chickpea yield is low and has stagnated. However, its very high prices compared to other crops encourage farmers to expand in area despite the low yield. Many biotic and abiotic factors contribute to these low yields.

The major abiotic factors identified are water and heat stress, whereas biotic factors are diseases, insects and weeds.

Fusarium wilt of chickpea is among the major constraints to increased and stable yields of chickpea in Sudan and many other countries. It was reported as an important fungal disease of chickpea in Sudan in River Nile State since early eighties (Freigoun, 1980a).The disease is the most important soil and seed borne disease of chickpea throughout the world (Haware and Nene, 1982; Jalali and Chand, 1992; Infantino *et al*., 2006).

In chickpea growing areas in Sudan,Fusarium wilt is among the most important diseases. The disease is especially serious in northern Sudan where chickpea is grown on stored soil moisture aftertherecessionoffloodwater of the Nile River and farmers in these areas did not adhere to crop rotations (Ali, 1996). The disease can cause a destructive damage to the crop and, hence reduces its production. Yield losses could reach 100% depending on varietal susceptibility and agro climatic conditions (Chand and Khirbat, 2009)

The causal organism of chickpea Fusarium wilt disease is *Fusarium oxysporum* Schlechtend. Fr. f. sp. *ciceris* (Padwick) Matuo and K. Sato (*Foc*)(Jalali and Chand, 1992; Haware, 1990; Nene and Reddy, 1987). The fungus could survive on crop residues in the soil for more than 6 years (Singh *et al*., 2007). The pathogen during this period undergoes different biological competition and environmental stresses which may lead to the existence of physiological races (Bendre and Barhate, 1998). To date, eight physiological races of *Foc* (0, 1A, 1B/C, 2, 3, 4, 5 and 6) have been reported (Haware and Nene 1982; Jim´enez-D´ıaz *et al.*, 1993a; Sharma and Muehlbauer, 2007) in different countries. Races 0, 1A, 1B/C, 5 and 6 are found in Mediterranean countries, India and USA, whereas2,3 and 4 were found in India. In Sudan, several *Foc* races were identified. Ali(1995)

found three unidentified races in River Nile State in north Sudan and named them 7, 8 and 9.In addition Suliman (2000) found in River Nile State two races that do not belong to any of the previously identified races and named them as10 and 11. Moreover,Kurmut (2002) identified a race similar to race 2 from the same state and reported the presence of an unidentified race from Shambat sick plot in Khartoum State.

In season 2013, the area grown in Gezira, central part of Sudan accounted to about 15000 ha (Gezira Scheme data). The expansion of chickpea production in these new areas provided that the source of seeds was primarily from areas with high fungus diversity.In the last few years, the disease emerged as a devastating and economicaly important constraint to chickpea cultivation. There is a lack of information about Fusarium wilt distribution and its population structure. Control of the disease by any means other than use of resistant cultivars will not be economiclly feasible. However, pathogens like *Fusarium oxysporum* f. sp. *ciceris* with high genetic diversity will hinder the effectiveness of resistant cultivars.Therefore, to develop an effective breeding program, knowledge of biotypes and genetic diversity of *Foc* is a pre- requisite. To assess genetic diversity morphological traits and molecular markers could be used. DNA based molecular markers have proven to be good tools to identify genetic variability in fungal pathogens. There is a high need for genetic diversity studies of the causal organism (*Foc*) as an aid in disease management and development of effective resistant varieties.

Therefore, this study was undertaken with the objectives to:

1/ Determine the distribution of Fusarium wilt disease in major chickpea growing areas of the Sudan.

2/ Identify the role of cultural practices done by farmers and weather conditions on disease incidence.

3/ Determinevariability amongisolates of *Fusarium oxysporum* f. sp. *ciceris* isolates causing chickpea wilt.

4/ Identify chickpea genotypes that are resistant to the key races of the pathogen (*Fusarium oxysporum* f. sp.*ciceris*)

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Chickpea

2.1.1. Crop History

The chickpea (*Cicer arietinum* L.) is the largest produced food legume in South Asia and the third largest produced food legume globally, after common bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.). It belongs to the family Fabaceae, subfamily Faboideae. Duschak (1871) traced the origin of the word to the Hebrew *'kirkes'*, where *'kikar'* means round. The word *arietinum* is also Latin, translated from the Greek *'krios'*, another name for both ram and chickpea, an allusion to the shape of the seed, which resembles the head of a ram (Aries) (Van der Maesen,1987). Chickpea is one of the earliest cultivated legumes: 7,500-year-old remains have been found in the Middle East. Chickpea is known with different namesthroughout the world,i.e. Chickpea (UK), Garbanzo (Latin America), Bengal gram (Indian), Hommes, Hamaz (Arab world), Shimbra (Ethiopia), Nohud and Loblebi inTurkey(Singh and Wakar, 1995)and Kabkabe in the Sudan.

The genus *Cicer* originated in South-eastern Turkey and spread to other parts of the world. It is adapted to relatively cooler climates and the largest area of adaptation is in the Indian sub-continent. In recent years,chickpea cultivation has spread worldwide (www.icrisat.org).

Chickpea is grown in over 45 countries in all continents of the world. Nearly 90% of the crop is cultivated asrainfed mostly on receding soil moisture and on marginal lands. It provides a high quality protein to the people in developing countries either by choice or because of economic reasons. In addition to having high protein content (20-22%), chickpea is

rich in fiber, minerals (phosphorus, calcium, magnesium, iron and zinc) and β-carotene (Gaur*et al*., 2010). Its lipid fraction is high in unsaturated fatty acids. Therefore, people in the developed countries consider it as a health food. Green leaves/twigs of chickpea are used in preparing a nutritious vegetable food in countries of South Asia. These green leavesare also used as high protein fodder mixed with cereal leaves. Chickpea Stover is fed to the cattle and goats as a nutrient-rich supplement to their major cereal fodder in the lean season (icrisat.org).

Chickpea plays a significant role in improving soil fertility by fixing the atmospheric nitrogen. It gets 80% of its nitrogen (N) requirement from symbiotic nitrogen fixation and can fix up to 140 kg N ha^{-1} from air (Gaur *et al*., 2010). Chickpea leaves gave substantial amount of residual nitrogen for subsequent crops and adds plenty of organic matter to maintain and improve soil health and fertility. Because of its deep tap root system, chickpea can withstand drought conditions by extracting water from deeper layers in the soil profile. If managed well, the crop could bring high returns to the farmer, in addition to enhancing sustainability of agricultural systems (Gaur *et al*., 2010).

In Sudan, chickpea is an important food legume and cash crop providing a great portion of the protein requirements for the population. The crop has a beneficial effect in the soil through fixing the atmospheric nitrogen and hence sustaining the productivity of the farming systems in the country. Chickpea wilt is a serious disease, that mostly prevalent in the dry weather conditions. It causes considerable yield losses in different chickpea cultivation areas depending upon its prevalence, intensity and environmental conditions.

2.1.2. Plant habit

Chickpea is a herbaceous annual plant which branches from the base. It is almost a small bush with diffused, spreading branches (Fig 1)

Fig. 1. General morphology of chickpea plants

2.1.3. Chickpea Types

Based on seed size and color, cultivated chickpea is grouped into the following two types (Cubero, 1975; Singh and Wakar, 1995):

1.Macrosperma *(Kabuli* type):The seeds of this type are large in size (100-seed mass >25 g), white or cream (beige)-colored seed with ram's head shape. They have thin seed coat and smooth seed surface (Fig. 2). The plant is medium to tall in height, with large leaflets and white flowers, and lack of anthocyanin pigmentation on the stem. Compared with Desi seeds, the Kabuli seeds gain higher levels of sucrose, lower levels of fibers and receive higher market prices.

2.Microsperma *(Desi* type):The seeds of this type are colored and have thick seed coat. The common seed colors include various shades and combinations of brown, yellow, green and black. The seeds are generally small and angular in shape with rough surface (Fig. 2). The plants are short with small leaflets and the flowers are generally pink in color and the plant shows various degrees of anthocyanin pigmentation. This type of chickpea accounts for 80-85 % of the chickpea cultivated areas mainly in India.

Fig.2. Desi and Kabuli chickpea types

2.1.4. Chickpea climatic requirements

Chickpea is a cool season crop and grown as a winter crop in the tropics and as a summer or spring crop in the temperate environments. Temperature, day length and availability of moisture are the three major a-biotic factors affecting flowering. In general, flowering is delayed under low temperatures and short days. Chickpea is sensitive to higher temperatures more than 35˚C and lower temperatures than 15˚C at the reproductive stage. Both extremes of temperatures cause flower dropping and reduction in pod set. There are wide variations in the agro climatic

conditions under which chickpea is grown around the world (Gaur*et al*., 2010).

Smithson *et al*.(1985) classified chickpea-growing areas into four major geographical regions (Indian subcontinent; West Asia, North Africa, and Southern Europe; Ethiopia and East Africa; The Americas and Australia)

2.1.5. Soil types suitable for the crop

Chickpea plants can be grown in different soil types like sandy, sandy loams and deep black soils. The best soils for chickpea growth are deep loams or silty clay loams devoid of soluble salt because such soils retain up to 200 mm moisture in the soil profile up to a depth of 1m (Saxena, 1987).Chickpea plants require good soil aeration, so that heavy soils require care in seedbed preparation.Chickpea does best on fertile sandy, loam soils with good internal drainage. Good drainage is necessary because even short periods of flooded or waterlogged fields reduce growth and increases susceptibility to root and stem rots.

2.1.6. Chickpea cultural practices

2.1.6.1. Land preparation

Land preparation for sowing chickpea is based on the soil type and cropping system. It is necessary to deep-plow the field at the beginning of the rainy season. This opens the soil deep and ensures efficient moisture conservation. Deep plowing also reduces wilting of chickpea that tends to develop due to the presence of hardpans in the root zone (Moolani and Chandra, 1970;Singh and Wakar,1995).

In the case of heavy soils, a rough seedbed must be prepared to avoid packing of the cloddy surface due to rains and to facilitate soil aeration and easy seedling emergence.

Chickpea plants are highly sensitive to poor aeration in the soil. Seedling emergence and plant growth are hindered if field surface is compact. Therefore, the field should have loose tilth and good drainage. The stubble and debris from the previous crop should be removed as these can harbor the pathogens that cause root diseases, such as root rot.

In Sudan, the crop was grown in the traditional cultivated areas of Wad Hamed area in North Sudan on stored soil moisture after the water of the River Nile subsides and this leads to high disease incidenceduringyearsof low flood (Ali *et al.*, 2002). In the newly cultivated areas of Gezira State, farmers grow the crop on ridges after ploughing and leveling the soils but recently they grow their crops on beds to avoid the excessive water during irrigations.

2.1.6.2. Seed treatment

Since *Fusarium oxysporum* f. sp.*ciceris* is a soil-borne fungus and can survive in the soil for long periods, it is not possible to control the disease through crop rotation. To protect the crop from seedling diseases, it is better to treat the chickpea seeds with fungicides that improve germination and seed yield without any adverse effects on nodulation. Treatment of chickpea seeds with a mixture of quintozene and thiram, each at 1.5 g kg^{-1} seed is recommended(Bhattacharyaand Sengupta 1984).The seed borne inoculum can be eradicated by seed dressing with Benlate T (benomyl30% + thiram30%) at 0.25% rate (Haware *et al.*,1978).

In Sudan, farmers do not treat the chickpea seeds with any kind of pesticides but they used to spray the crop with insecticides to control insect pests such as leafhopperand pod borers. Results of experiments done by scientists for seed treatment against Fusarium wilt disease indicated thatthe tested Tecto-TM and Quinolate Profungicidesimproved the chickpea seedling emergencebut neither significantly decreased the final incidence

of dead plants, or increased the grain yield (Ali *et al*., 2002). Apron star at a rate of 2.5g/kg of chickpea seeds was also used and gave good results in improving crop stand and controlling chickpea chloratic dwarf viral disease(Mahir*et al.*, 2007).

2.1.6.3. Sowing methods and dates

In India, West Asia, and North Africa, farmers use traditional plows with an attached V-shaped funnel for sowing. Wider row spacing (45–60 cm) can be used in large seeded Kabuli chickpea and irrigated crops which are expected to have greater plant width (Gaur*et al.*, 2010).Broad bed and furrow system or ridge and furrow system are very useful for irrigation and drainage. The seeds are dibbled on these ridges5-8 cm deep to contactthe moist soiland to enhance seedlings emergence.Row-to-row spacing of 30 cm and intra row spacing of 10 cm are generally usedto give a plant population of about 33 plants m^{-2}i.e. 330,000 plants ha^{-1} (Gaur*et al.*,2010).

In Sudan, chickpea wasgrown in the traditional production areas of Wad Hamid basin and Rubatab area in northern parts of the country. In these areas, chickpea was grown on stored soil moisture after the floodwaters of the Nile River subside (Ali, 1996). The chickpea grown was small Kabuli type that broadcasted after irrigation and land preparation.The irrigation frequency after sowing varied from nil to frequent irrigations at 10-day intervals.Two sowing methods (on-ridge andon flat), were studied at Hudeiba Research station, River Nile State and the results revealed that sowing on-ridges was superior to sowing on-flat. The optimum sowing time was found to be mid-November (Ibrahim,1996).

After the expansion of chickpea growing areas to the central parts of the country as an irrigated crop, chickpea is sown on ridges or wide beds. The ridges are either freshly made or left over from the previous season.

The crop is sown as a winter crop in November- December and midNovembersowings are optimum (Ibrahim, 1996). December sowings were consideredas late sowings, but sometimes it is beneficial when it is cooler as the crop may avoid the higher temperatures of November and thus the leafhopperinsects that transmit the chickpea chloratic yellow dwarf virus.

2.1.6.4. Seeding rate

Seeding rate of chickpea differs from variety to variety, depending on seed size. The seeding rate for small size chickpea types (less than 20g) is 50-60kg/ha, for medium types (20-30g) 60-90kg/ha, for large seed size (30-40g) 90-120kg/ha and for extra large sizes (more than 40g) 120-150kg/ ha are used(Gaur *etal.*, 2010).In Sudan,studies revealedthat plant population of 33 plants/m^2 or a seed rate of60 kg/ha were satisfactory for good crop establishment andconsequently for maximum grain yield (Ibrahim,1996),but some farmers may use lower or higher seed rates.

2.1.6.5. Fertilization and Inoculation

Chickpea is a pulse crop, and has the ability to fix 60-80 per cent of its nitrogen requirement from air in the soil under optimum conditions. Under good growing conditions, chickpea is considered a relatively good nitrogen-fixer, but if nitrogen fixation is not optimized due to growing conditions or low levels of nitrogen fixing bacteriaInoculation with appropriate strain of nitrogen-fixing*Rhizobium* is required (Singhand Wakar, 1995). Chickpea has a very specific relationship with Rhizobium and it is essential to use an inoculant specifically developed for chickpea. Inoculants for pea and lentil will not produce nodules on chickpea and are not suitable. In Sudan,the effects of fertilizer (N and P) application and *Rhizobium* inoculation onyield performance ofchickpea were studied over different seasons and locations. Results revealed that the response to P was

negligible andgrain yield response to N fertilization was inconsistent, but indicated the needof a starter dose (10-20 kg N/ha).The *Rhizobium* inoculation studies showed that the local strains were quite effective(Ibrahim,1996). In Gezira, fortunately,as the soil is very rich of Rhizobium bacteria,farmers do not use any kind of inoculants.

Superior seed quality is needed for successful chickpea production. Seedsshould be tested at an accredited seed-testing laboratory to determine percentage of germination, seed health and chickpea seed purity.

2.1.6.6. Watering

Chickpea is generally grown as a rain fed crop. The reproductive stage is the most sensitive stage to moisture stress developed through expanded irrigation intervals,so that two irrigations, one at branching and the other at pod filling stages are important for higher yields **(**Gaur*et al.*,2010**)**. As chickpea growth is indeterminate, higher number of irrigations may lead to excessive vegetative growth in heavy soils. Kabuli chickpea should never be irrigated excessively after sowing, particularly in deep black soils and this is because the Kabuli chickpea seeds have thin seed coat and deteriorate faster as compared to Desi type and are also more susceptible to seed rot and seedling damping off**(**Gaur *et al.*,2010**)**.

The majority of chickpea farmers in Sudan irrigate their cropsat two-week intervals with sufficient irrigations. Frequentirrigation (7-10 day intervals) during the whole crop cycle always resulted in the highest grain yield(Ibrahim, 1996)

2.1.6.7. Weed management

Weed problems have proven to be a major constraint to successful chickpea production as the crop is a poor competitor with weeds at all stages of growth. Therefore, weed managementmust be carefully planned and implemented. The most important considerations are an assessment of

the history of weed infestation on a field before planting chickpea. Fields containing large weed seeds reserves in the soil due to recent or past weed problems should be avoided. Application of pre emergence herbicides was found effective in controlling early flush of weeds and, hence improve crop productivity(Corp*et al.*, 2004).For weed management of chickpea in Sudan farmers tend to do hand weeding, as it is necessary. The production of legumes in northern Sudan is greatly constrained by weeds. The traditional methods of weed control in northern Sudan involve late hand weeding done voluntarily by animal owners for collecting fodder for livestock. A series of experiments on weed management in cool-season legumes werecarried out in different parts of northern Sudan from 1985 to 1995. Unrestricted weed growth and delayed weeding reduced seed yield of chickpea by up to 80% (Mohamed, 1996). Results of spraying pre-emergence herbicide and herbicide mixtures on chickpea fields showed that Pursuit, Igran and Gesagard (in a tank-mixture with Stomp or Goal) gave adequate control of weeds and increased the grain yield of chickpea. However, in some locations, the herbicide (Pursuit) was phytotoxic to chickpea (Mohamed, 1996)

2.1.7. Chickpea production

Chickpea (Cicer *arietinum* L) is an important food legume grown for domestic purposes. It is mainly produced and consumed (95%) in developing countries. In 2010 cropping season, it was cultivated in about 11.9 million hectares. Chickpea production has increased over the past 30 years from 6.6 to 10.0 million metric tons. Most chickpeasare grown in South Asia, which accounts for more than 75% of the world chickpea area. India is by far the largest chickpea producing country. Over the period 1978 to 2010, the area under chickpea in India increased marginally from 7.6 to 7.9 million hectares, but production increased by 40% from 4.8 to

6.8 million metric tons. Other important chickpea producing countries are Pakistan, Iran, Turkey, Mexico, Canada,Ethiopia, Myanmar and Australia (cigar.org).

Despite being a crop of the temperate regions, advances in plant breeding have enabled chickpea cultivation to gradually spread to the sub-tropical and tropical regions of Africa, North America and Oceania. In Africa, it is grown mainly to utilize fallow lands. Africa's share of the global chickpea area has gradually increased to 4.7% in 2008–2010 from 3.8% in 1981–83(cigar.org).

In Sudan according to FAOSTAT(2012), the chickpea production increased from 2000 tons in early 1990s to 16000 tons during the past two decades. In the 1990s, the crop was introduced to the central states of Sudan, especially the Gezira where the area gradually increased to reach about 8000 ha in 2010(Fig. 3). In 2013 cropping season, chickpea was cultivated on 15000 ha of land in Gezira scheme alone with productivity between 0.8-2.8 tons/ha depending on cultivar, management and weather conditions(Gezira Scheme data; Khalifa, 2013- personal communication)

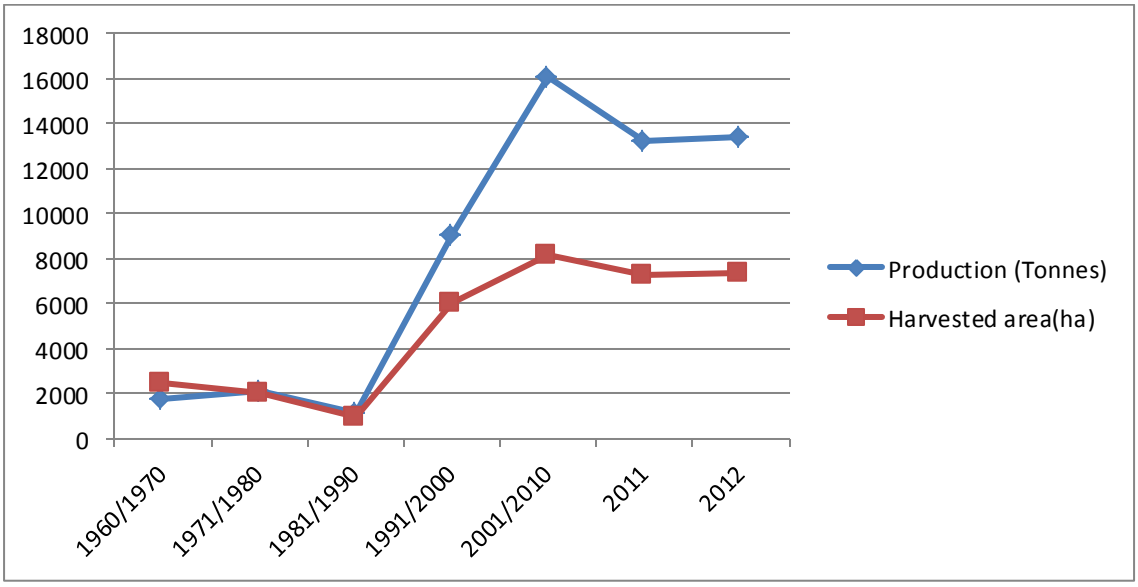


Fig. 3. Average chickpea harvested area (ha) and production (tons) in Sudan from 1960 to2012

Despite its economic importance and strong national and international breeding programs, globally, the productivity of chickpea has not improved considerably over the years. Chickpea potential seed yield of about 5 t ha^{-1} has been reported and the realized seed yield of 850 kg ha^{-1} is a result of lack of widely adapted cultivars and susceptibility to several biotic and a biotic stresses (icrisat.org).

2.1.8. Biotic and abiotic stresses on chickpea

Major constraints in realization of the full yield potential of chickpea include various abiotic and biotic factors. The wide gap between average yield and potential yield is mostly due to diseases, pests and poor management practices. Among the abiotic stresses, drought is the most important stress in chickpea since the crop is mostly grown on rain-fed marginal lands (Singh *et al.,* 1994). The crop invariably suffers from moisture stress at one or the other stages of development depending on water availability in the soil. Cold is the second most important abiotic stress and susceptibility to cold is greater at the late vegetative stage than at theseedling stage (Singh *et al.,* 1994).

Among the causal agents of biotic stresses, about 67 fungi, 3 bacteria, 22 viruses and 80 nematodes have been reported on chickpea (Nene *et al.,* 1996), but only few of these cause economically important diseases (Haware, 1998). The insect *Helicoverpa armigera* which feeds on foliage, flowers and developing seeds, is the most important insect pest of chickpea (Ranga Rao*et al.,* 2013) while chickpea yellow stunting is the most serious and prevalent viral disease in most chickpea growing regions of the world (Horn *et al.,* 1996). The most economically important fungal diseases of chickpea are wilt/root complex, foliar diseases like Ascochyta blight and Botrytis grey mold. Wilt and Ascochyta blight are the most

devastating diseases affecting chickpea in tropical and temperate regions, respectively, but in Sudan wilt/root rot and viruses are the key biotic constraints to chickpea production.

2.2.Fusarium wiltcausing pathogen

Fusarium is a large genus of filamentousfungi widely distributed in soil and in association with plants. Most *Fusarium* species are soil-borne fungi and have a worldwide distribution. Some are plant pathogens causing root and stem rot, vascular wilt or fruit rot. Other species cause storage rot and are important mycotoxin producers. Several species, notably *F. oxysporum*, *F. solani* and *F. moniliforme,* are recognized as being pathogenic to man and animals.*Fusarium oxysporum* Schlechtend.:Fr.f.sp.*ciceris* (Padwick) Matuo &K.Sato is the causal pathogen of fusarium wilt in chickpeas.The fungus is a common soil inhabitant and produces three types of asexual spores.Microconidiawhich are ellipsoidal and either have no septum or a single one and formed from phialides in false heads by basipetal division. Macroconidia which are straight to slightly curved, slender, thin walled, usually with three or four septa with a foot-shaped basal cell and a tapered and curved apical cell. Chlamydosporesare globose and have thick walls. They are important as endurance organs in soils where they act as inocula in primary infection (Leslie and Summerell, 2006).

There are distinctvariations in colony characteristics of the fungus such as color, shape and texture. *In vitro* studies were conducted on different culture media, temperature and pH levels on mycelial growth of *Fusarium oxysporum*f. sp. *ciceris* isolates in Bangladesh (Islam *et al.*, 2012). The results showed that colony colors were deep pink, pinkish white, whitish pink and White. Pigmentations such as pink, violet and brown were observed. The intensity of colony colour changedby time depending on the period of incubation and the colony shape was irregular

round and slightly round, with colony texture loose, compact and slightly compact. The results revealed that Potato dextrose, Czapek's Dox agar media were better for the radial growth of *Fusarium oxysporum* isolates with a mean maximum and minimum growth of 90mm and 63.5mm, respectively (Islam *et al.*, 2012).Similar studies were conducted by Khilare and Rafi (2012) in India and they found that the best growth of the fungus was on Czapek dox agar and PDA media among the six tested culture media.

2.3 Fusarium wilt disease

2.3.1. Distribution

Fusarium wilt of chickpea is prevalent in almost all the chickpea growing areas around the globe. The disease has been reported from several countries including India, Bangladesh, Burma, Ethiopia, Mexico, Pakistan, Syria, Tunisia, Chile, Iran, Nepal, Sudan, the United States, Peru, USSR, Malawi, Spain, Turkey and Italy. Nene and Reddy (1987) recorded a distribution of *F. oxysporum* f. sp. *ciceris* covering North America, Europe, Middle East, Asia and South East Asia.

Fusariumwilt caused by *Fusarium oxysporum* f sp *ciceris*is a serious fungal disease of chickpea (Khan, 1980). The fungus is a common soil inhabitant and produces three types of asexual spores (macro conidia, micro-conidia and chlamydospres). The disease is especially important in low rainfall areas, where weather conditions are favorable for disease development. It is a major constraint to chickpea production in many countries covering North America, Europe, Middle East, Asia and South East Asia.(Neneand Reddy, 1987; Haware, 1990; Jalali and Chand, 1992) and reported in 33 countries worldwide (Nene*et al.*, 1996). The disease causes an average of 10–15% annual yield losses (Singh and Dahiya, 1973) which may reach 100% depending upon the environmental conditions

(Grewal and Paul, 1970; Chand and Khirbat, 2009). The pathogen is highly variable in its cultural characteristics andpathogenicity. Complete loss of the crop may occur depending on varietalsusceptibility and agroclimatic conditions (Chand and Khirbat, 2009).

The disease is more prevalent in the Indian subcontinent, United States, Tunisia, Turkey, Ethiopia, Spain, Mexico and the Middle East (Halila and Strange, 1996; Nene *et al.*,1989; Westerlund *et al.*,1974).

The disease isprevalent in Sudanand was reported in the early eighties by Freigoun (l980a) who reported that*Fusarium oxysporum* is the causal agent of wilt in Chickpea.Fusarium is a large genus of filamentousfungi widely worldwide distributed in soil and in association with plants. The genus includes a number of economically important plant pathogenic species like *F. oxysporum* which have been studied for more than 100 years causing root and stem rot, vascular wilt and fruit rot. Plant pathogenic *F. oxysporum* strains have a broad host range and individual isolates usually cause disease only on a narrow range of plant species.

2.3.2. Disease Symptoms

The primary infection of Fusarium wilt is through chlamydospores or conidia. Following infection of host roots, the fungus crosses the cortex and enters the xylem tissues. It then spreads rapidly up through the vascular system, becoming systemic in the host tissues, and may directly infect the seed. Discoloration of the internal tissues progresses from the roots to the aerial parts of the plant, yellowing and wilting of the foliage occur, and finally the plant may die. Lentil, pigeon pea and pea were identified as symptomless carriers of the pathogen (Haware and Nene, 1982). Soil temperature and moisture may affect the appearance of symptomsof the disease whichare influenced by different chickpea cultivars (Landa *et al.*, 2006).Wilting in chickpea can be observed 20-25 days after sowing. The

disease appears at seedling and reproductive stages of the crop under field conditions. The infected plants do not show external rotting and look healthy, but their roots when splitted vertically from the collar region downward, show brown discoloration of the internal tissues. The main symptoms of the disease are drying and yellowing of leaves from the base upward, browning of vascular bundles, improper branching, drooping of petioles, withering and wilting of plants(Chand and Khirbat, 2009; Prasad and Padwic, 1939;Westerlund *et al*,1974 and Nene and Haware,1980).Early wilting reduced the seeds number/plant and caused more yield losses than late wilting (Haware and Nene, 1980). The seeds harvested from wilted plants are lighter, wrinkled and duller than those from healthy plants.

Following infection of host roots, the fungus crosses the cortex and enters the xylem tissues. It then spreads rapidly upwards through the vascular system, becoming systemic in the host tissues, and may directly infect the seed. The root tips of healthy plants growing in contaminated soil are penetrated by the germ tube of spores or the mycelium. Entry is either direct, through wounds, or opportunistic at the point of formation of lateral roots. The mycelium takes an intercellular path through the cortex and enters xylem vessels through the pits. The pathogen is primarily confined to the xylem vessels in which the mycelium branches and produces microconidia (asexual spores of the fungus). The microconidia detach and are carried upward in the vascular system until movement is stopped, then they germinate and the mycelium penetrates the wall of the adjacent vessel. The water economy of infected plants is eventually severely compromised by blockage of vessels, resulting in stomatal closure, wilting and death of leaves, often followed by death of the whole plant. The fungus then invades all tissues of the plant, to reach the surface where it sporulates profusely. Spores may then be dispersed by wind, water or movement of soil or plant

debris. *F. oxysporum* f. sp. *ciceris* can survive as mycelium and chlamydospores in seed, soil and also on infected crop residues, roots and stem tissue buried in the soil for up to 6 years (Singh *et al.*, 2007; Cunnington*et al.,* 2007).

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, which progress from the petioles and younger leaves in two or three days to the whole plant. The older leaves develop chlorosis, while the younger leaves stay dull green. At a later stage of the disease, all leaves turn yellow. Discoloration of the pith and xylem(Fig 4, B)occurs in the roots and could be seen when they are cut longitudinally(Cunnington*et al.,* 2007)

Fig. 4. Infection with the disease in the field (A) and discoloration of the vascular system (B)

2.3.3. *Fusarium oxysporum* f. sp. *ciceris* life cycle

Jalali and Chand (1992) summarized the life cycle of the fungus in Fig.5 below.

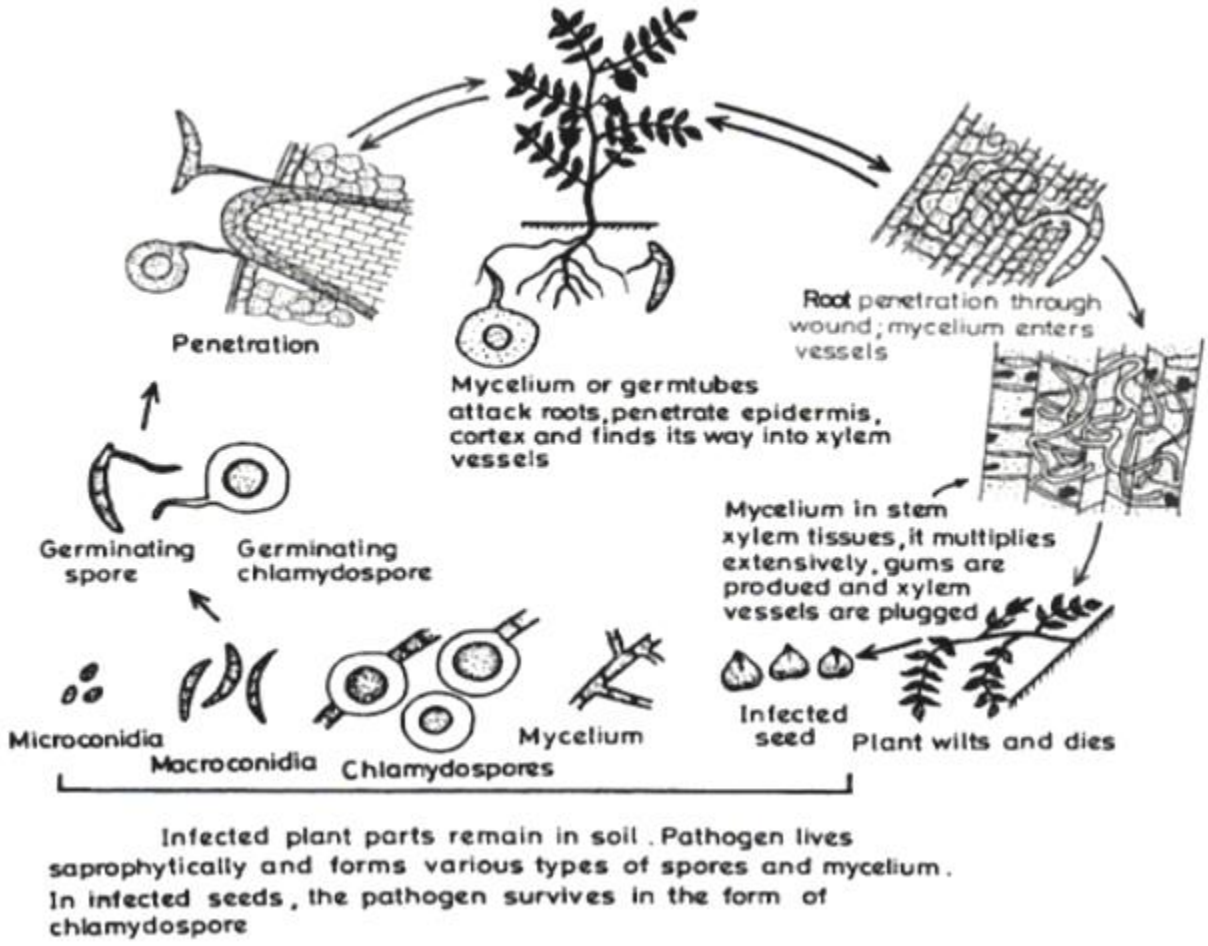


Fig. 5. Disease cycle of *F. oxysporum* f. sp. *ciceris* (Source: Jalali and Chand,1992)

2.3.4. Host range of the pathogen

The host range of *Fusarium oxysporum* f. sp. *ciceris* includes *Cicer arietinum* (chickpea), *Cajanus cajan* (pigeonpea), *Lens culinaris* ssp. *culinaris* (lentil) and *Pisum sativum* (field pea) (Haware and Nene, 1982; Hawaree*t al.*, 1986; Nene *et al.*, 1996).

Symptomless plants provided inoculum to infect and produce a visible disease in healthy plants when favorable conditions for disease development resumed. Soil-borne pathogens specific to a few hosts survives in soil either in the debris of their specific hosts or of symptomless carriers. *Fusarium oxysporum* f sp *ciceris* is pathogenic only to *Cice*r spp., but it also colonized roots of lentil, pea and pigeonpea. These three crops

(lentil, field pea and pigeonpea)are considered symptomless carriers because the pathogen was isolated from their roots. Colonization of these plants roots by *Fusarium*. *oxysporum*. f. sp.*ciceris*could be artificially demonstratedand no symptoms were seen on the three crops. This information could be useful in planning crop rotations involving chickpea andplanting crops that do not allow colonization by *F*. *oxysporum* f sp *ciceris* in rotation with chickpea is expected to reduce the inoculum level in the soil(Haware and Nene, 1982).

2.3.5. Pathogen transmission and survival

The pathogen could be dispersed through infested plant debris like roots, leaves and stems and through contaminated soils and seeds. The principal means of dispersal of the pathogen over short distances is by water or contaminated farm equipments. Conidia can be dispersed by water flow, rain-splash and by movement of infected soil or plant material. Over longer distances, the pathogen may be dispersed in infected plant debris, seeds and chlamydospores in associated soils.Once in an area, *F*. *oxysporum* f. sp. *ciceris* survives between crops in infected plant debris as mycelium, microconidia, and macroconidia and most commonly as chlamydospores. The pathogen is able to survive for many years either in soil as chlamydospores or as a saprobe in plant debris (Cunnington *et al.*, 2007)

2.3.6. Physiological races of the pathogen

*Fusarium.oxysporum*f. sp. *ciceris*, exhibits great diversity. Two pathotypes of the fungus were distinguished based on distinct yellowingor wilting symptoms that they cause in chickpea plants (Trapero and Jiménez-Diaz, 1985). The yellowing pathotype induces progressive foliar yellowing with vascular discoloration, followed by plant death within 40 days of

inoculation. The wilting pathotype induces severe chlorosis and flaccidity, vascular discoloration, and plant death within 20 days after inoculation. In addition to variation in symptom type, there are eight races of *F. oxysporum* f. sp. *ciceris* (races 0, 1A, 1B/C, 2, 3, 4, 5, and 6), which are identified by reactions on a set of differential chickpea cultivars (Haware and Nene, 1982).

Races 0 and 1B/C induce the yellowing syndrome (yellowing pathotype), whereas races 1A, 2, 3, 4, 5, and 6 induce the wilting syndrome (wilting pathotype) (Jiménez-Díaz *et al.*, 1993a). The eight races have distinct geographic distributions. Races 2, 3, and 4 have been reported only in India (Haware and Nene, 1982), whereas races 0, 1B/C, 5, and 6 are found mainly in the Mediterranean region and in the United States (California) (Halila and Strange, 1996; Jiménez-Díaz *et al.*,1993a; Jiménez-Gasco *et al.*,2001). Race 1A has been reported in India (Haware and Nene, 1982), California, and the Mediterranean region(Jiménez-Díaz *et al.* 1993a, Jiménez-Gasco *et al.*, 2001).

2.3.7. Disease management

Disease control is quite difficult because the pathogens survive in infected soil for many years, even in the absence of the host plant. Therefore, the most practical, effective and economical method of controlling Fusarium wilt of chickpea is through the use of resistant cultivars(Nene andReddy, 1987; Haware *et al.*, 1992), the effectiveness of which is curtailed by the occurrence of pathogen races(Jimenez-Gasco *et al.*, 2004).

Management practices directed towards pathogen for checking the progression of the disease occurrence could be exclusion and eradication of the pathogen to reduce its inoculum. By the varied nature of pathogen involved, evolving resistant varieties has so far proved to be the best bet,

although other conventional chemical, cultural methods and biological control have also yielded good results. Since this crop is grown principally in rain-fed areas, many of the known conventional chemical methods have not found wide adoption (Chand and Khirbat, 2009). However, the adequate assessment of such control methods for its effectiveness in the management of Fusarium wilt of chickpea requires a better understanding of the epidemic development of the disease.

2.3.7.1. Cultural practices

Early planted crops usually attract more disease. The studies of Navas-Cortés*et al.* (1998) showed that for each year of experiment, epidemic development of chickpea Fusarium wilt was related mainly to the date of sowing .The lower disease incidence in late-sown crop was considered to be due to low temperature prevailing during the period of late-sown crop.

Plants spaced at 15-20 cm had much higher disease incidence than those spaced at 7.5 cm; this was attributed to the shallower root system in widely spaced plants which were susceptible to wilt when subjected to moisture stress (Bahl, 1976).Singh and Sandhu (1973), found thatplanting of seeds at proper depth (10-12cm) using lower seed rate helped to minimize disease, while shallow sown crop seemed to attract more disease(Sugha *et al.*, 1994a). Development of wilt is more prominent under moisture stress conditions. Chand *et al.*(2009) stated that, the disease is more severe in light sandy soil than heavy clay soils and high soil temperatures and deficiency of moisture appear to have a definite bearing on the disease incidence and delaying sowing dates helps in minimizing the disease. The amount of organic matter is inversely related to wilt incidence, i.e. development of wilt is favored by increase in nitrogen content of the soil. The optimum temperature and pH for pathogen growth are 25ºC and

5-6.5 respectively. Mixed cropping of chickpea with wheat and berseem(*Trifolium alexandrinum* L.), gave measurable disease control(Chand and Khirbat,2009).

In Sudan, the disease is especially serious in the traditional production areas of Wad Hamid basin in northern Sudan, where chickpea is grown on stored soil moisture after the flood waters of the Nile River subside (Ali, 1996; Faki *et al.*, 1996). In these areas, farmers do not practice crop rotation and the crop at the post-flowering stage is often subject to moisture stress in years of low flood. Taha and Ali (1991), reported that the incidence of wilt/root-rots at Hudeiba (River Nile State) was significantly higher in flat plantingas compared to ridge planting, whereas at Rubatab (River Nile State) the difference between thetwo sowing methods was not significant.At both locations, the highest disease incidence was observed on the chickpea crop in which the irrigationwater was stopped 50 days after sowing.

Recently, the crop is introduced to the central parts of the country, with heavy clay soils, as an irrigated crop. The crop is also successfully grown in Hawata area in eastern Sudan and Jebel Marra in western Sudan (Faki *et al.,* 1992). The great expansion in the cultivated areas of the crop requires more attention and efforts for transferring ideal cultural practices technologies to farmers to avoid the disease and obtain higher yields

2.3.7.2. Chemical control

Treatment of chickpea seeds with protectants or systemic fungicides was reported to increase seedling emergence and to reduce pre-emergence damping- off (Verma and Vyas, 1977; Kotasthane and Agrawal, 1978; Shukla *et al.*, 1981; Jiménez-Díaz and Trapero-Casas, 1985). The seed-borne inoculum can be eradicated by seed-dressingfungicides, Benlate 1.5%, Benomyl 30% + Thiram 30% (Haware *et al.*, 1978). Nikam *et al.*,

(2007) found Thiram (0.15%) + Carbendazin (0.1%) to be effective against *Fusarium* wilt. Christian *et al.*(2007)who studied the effect of several fungicides against chickpea Fusarium wilt reported high inhibition of the fungus by Carbendazim, Benomyl and Captan.

Ayyub (2001) evaluated eleven fungicides against chickpea Fusarium wilt and found that Benlate, Folicar and Derosal are the most effective against mycelial growth of the fungus. He observed moderate response toTopas-l00 and Tilt, whereas Daconil, Antracol, Apron and Polyram combi in these studies were found least effective.

The use of seed dressing fungicides although has helped increase of chickpea yields but constant use of chemicals can induceresistance in target organisms and contaminate the environment with very toxic substances. In addition, cost of fungicides is high and cannot be afforded by famers in many countries.

In Sudan, Ali (2002) conducted two field trials to verify the efficacy of the seed-dressing fungicides Tecto-TM and Quinolate Pro to control Fusarium wilt. One trial was carried out in the wilt-infected plotand the other in farmers' fields with a history ofhigh disease incidence. In both trials, the two seed-dressing fungicides significantly increased seedling emergencein the wilt-infected plot, but neither significantly decreased the final incidence of dead plants nor increased the grainyield.

Hamed (2012) conducted an experiment at Gezira Research Farm and some farmers' fields to verify the efficacy of the seed dressing fungicide Apron Star (2.5g/kg of seeds) to control the post emergence diseases of chickpea. He found that the fungicide significantly increased the vegetative growth of the crop and decreased the insect pests and controlled the viral diseases compared with the untreated control treatments, but did not decrease the final incidenceof wilting plants.

2.3.7.3. Biological control

Disease management using pesticides, especially in legumes adversely affect the beneficial microbial populations present in the ecosystem. Considering these deleterious effects of synthetic pesticides, plant extracts as alternative agents for management of pathogenic microorganisms gave effective results.

Plant extracts from different plant parts became valuable antifungal agents to control pathogens. These natural products have no obvious health and environment hazards and can be easily prepared by farmers.Shukla and Dwivedi (2012) studied *in vitro* the efficacy of Turmeric, Garlic and Black pepper plant extracts to control *Fusarium oxysporum* f.sp.*ciceris,* the causal agent of chickpea Fusarium wilt. All the plant parts extracts inhibitedthe growth of the pathogen at15% concentration of Garlic, Turmeric and Black pepper inhibited the growth of the pathogen by 94.63%, 87.96% and 77.74% (at $p< 0.01$), respectively.

Singh *et al*. (1979) observed that when seeds of chickpea (*Cicer arietinum*) treated with an aqueous garlic leaf extract and grown in soil infested with *F. oxysporum* f.sp. *ciceris* and *Sclerotinia sclerotiorum*, the resulting seedlings were wilt-free, whereas untreated seeds resulted in seedlings with wilt symptoms. Chand and Singh (2005) reported that seed treatment with bulb extract of *Allium sativum* reduced chickpea wilt by 42% compared with the untreated control. Neem oil is also significantly superior in reducing chickpea wilt incidence(Chand and Singh, 2005). Leaf extract of *Azadirachtaindica* at 100% concentration completely inhibited germination of the pathogen spores (Singh and Chand, 2004).

Biocontrol agents such as *Trichoderma* spp., *Bacillus* spp. and fluorescent *Pseudomonas* gave measurable reduction in disease progress.Merkuz and Getachew (2012) studied *in vitro* the effect ofthirty-eight *Trichoderma* isolates collected from Northwestern Ethiopia on colony

growth of *Fusarium oxysporum* f.sp. *ciceris.* He found that sixteen isolates inhibited the growth of the pathogen, seventeen isolates showed mycoparasitic effects and five isolates showed lysis effects on *F. oxysporum* f.sp. *ciceris.*Merkuz and Getachew (2012) also studied the effect of seed dressingof two local chickpea varieties with the sameisolates against chickpea wilt in the glass house and heobserved significant differences among the treatments in reducing wilt incidence on the two local varieties. Significant differences were also recorded in fresh and dry weight of shoots in thetreated local varieties compared with the un treated control.

Moradi*et al.,* (2012) also studied the effect of *Bacillus subtillis* and *Trichoderma harzianum*, in commercial formulations alone or in mixture, on glucanassoluble protein content, β-1, 3-glucanase enzyme activity and suppression of *Fusarium* wilt disease in two chickpea cultivars. They found that the disease severity was significantly reduced by *B. subtillis, T. harzianum* and their mixtures by 40% compared to the untreated control. *B. subtillis and T. harzianum,*either singly or in combination in both seed and liquid inoculation methods,effectively suppressed the disease and increased the protein content and β-1, 3-glucanase enzyme activity,whicheffectively improved the resistance to *Fusarium* wilt disease.

2.3.7.4. Resistant cultivars

Resistant cultivars are one of the few and the most effective means for managing Fusarium wilt of chickpea (Jiménez-Díaz *e. al.,* 1993b; Landa *et al.,* 2004; Landa*et al.,* 2006). However, their deployment has not been extensive because of poor agronomic characteristics in some developed cultivars. Furthermore, the high pathogenic variability in *F. oxysporum* f. sp. *ciceris* populations may limit the effectiveness and extensive use of available resistance. Sources of resistance against *F.*

oxysporum f. sp. *ciceris* have been identified (Sharma*etal*, 2005; Sharma and Muehlbauer, 2007) and exploited in several chickpea breeding programs consequently. Fair number of resistant chickpea germplasm lines operative against specific races of the pathogen have been developed. Adequate characterization of the resistance of chickpea lines and cultivars to specific races of *F. oxysporum* f. sp. *ciceris* is essential for resistance deployment. In the absence of agronomical and/or commercially suitable resistant cultivars, prediction of disease risk potential in a geographic area based on assessment of pathogen race and inoculum density thresholds in soil and susceptibility of cultivars can be of use for the management of Fusarium wilt in chickpea (Navas-Cortés*etal.*, 2007). For instance, advancing the sowing date of moderately susceptible cultivars from early spring to early winter can contribute to control the disease in Mediterranean environments.These benefits could be overridden if high inoculum density or a highly virulent race of the pathogen prevails in the soil (Landa *et* al, 2004;Navas-Cortés*et al.*, 1998).

In Sudan,chickpea variety improvement was started in the early seventies, in collaboration with the International Center for Agricultultural Research in the Dry Areas (ICARDA) and the International Crops Research Institute for the Semi- Arid Tropics (ICRISAT). Large number of germplasm lines were introduced and by a huge efforts, between breeders and pathologists, extensive screening and selection methods were done and a number of lines emerged and tested (Sheikh Mohamed, 1996; Ali *et al.*,2002).Selections were for adaptation, high and stable yield, resistance to wilt/root-rotdisease, earliness and large seed size. The evaluation justified the release of the kabuli type line NEC 2491(lLC 1335)in the year1987 to be grown by farmers in northern Sudan under the name Shendi-I. On-farm evaluation of some chickpea lines also justified the release of the line ILC 915 in 1993 to be grown by farmers in the Sudan

under the name Jebel Marra-I (Sheikh Mohamed, 1996). The ongoingevaluation of large-seeded and medium-seeded kabuli chickpea lines underfarmer conditions in the same locations showed promising genotypes releasedin 1996. These genotypes wereICCV-89509, Flip -89 82Cand ICCV-2. They were recommended to be grown by farmers in the Sudan under the names Atmour, Salawa and Wad Hamid, respectively,as largeseededcultivars. Many promising medium- and large-seeded genotypes with50-100% higher grain yield and seed size over the checks (Shendi and Jebel Marra) were released in 1998 such as ICCV-91302 (Burgieg), ICCV-92318 (Hawata) and Flip 91-77C(Matama)(Table 1). The line ICCV-2 showed wilt/root-rot disease resistance and good performance under residual moisture conditions (Ali *et al*., 2002; Sheikh Mohamed,1996; ICRADA,2003). The reaction of these cultivars to wilt/root rot disease is shown in Table (1).

Table 1. Chickpea released cultivars in Sudan from 1987 to1998

Cultivar Name	Arabic Name	Accession No.	Year of release	Reaction to wilt diseaseat (HRS)
Shendi	شندى	ILC1335	1987	HS
JabelMarra	جبلمره	ILC 915	1993	HS
Atmuor	عتمور	ICCV-89509	1996	MS
Wad Hamid	ودحامد	ICCV-2	1996	HR
Salawa	سلوه	Flip 89-82C	1996	MS
Burgeig	برقيق	ICCV-91302	1998	HR
Hawata	حواته	ICCV-92318	1998	R
Matama	متمه	Flip 91-77C	1998	MS

HS=Highly Susceptible;MS=ModeratelySusceptible; R=Resistant; HR=HighlyResistant. HRS=Hudeiba Research Station

2.4. Pathogenic variability and race identification

The identification of pathogenic races of *F. oxysporum* f.sp. *ciceris* in a given area is important for disease resistance breeding programs and for the efficient use of resistant cultivars. The classical method of race identification involves inoculation of differential chickpea cultivars with a particular *F. oxysporum* f. sp. *ciceris* isolate and determining its pathogenicitybut the morphological identification of the fugus races is not easy to assess.

A lot of work had been done on pathogenic variability of *Fusarium oxysporum* f. sp. *ciceris* by many scientists (Haware and Nene, 1982; Jim´enez-D´ıaz *et al.*, 1993a; Kelly *et al.*, 1994).

Jim´enez-Gasco *et al* (2001)characterized 29 isolates by pathogenicity tests on a set of differential cultivars and found that these isolates belonged to yellowing and wilting pathotypes and identified them with molecular markers as races 0, 1B/C, 5 and 6.

Fusarium oxysporum f. sp. *ciceris* isolates representing eight provinces located in four regions of Turkey were analyzedfor pathogenic variability on a set of differential chickpea cultivars. The isolates were identified as races 0, 2 and 3 (Bayraktar *et al.*, 2012).

In Sudan, variability among populations of *F. oxysporum* f. sp. *ciceris*, thechickpea wilt pathogen was studied by Ali (1995) at Hudeiba Research Station, River Nile State. Based on the reactions of ten differential chickpea cultivars to six isolates of *F. oxysporum* f. sp. *ciceris* obtained from Hudeiba Research Farm (HRF-l, HRF-2, HRF-3 and HRF-4), Wad Hamid (WH) and Rubatab (Rub), the isolates were grouped into three races (pathotypes), designated as race 7, 8 and 9. These races are distinct from races 1,2,3 and 4 identified by Haware and Nene(1982)in India; races 0and5identified by Cabrera de la Colina *etal* (1985) in Spain and 6identified in Calafornia by Phillips(1988)(Ali, 1996).Suliman (2000) identified races 10 and 11 fromthe same areas in River Nile State.

Kurmut (2002) collected and studied the effect of some chickpea wilted isolates of *Fusarium oxysporum* f. sp. *ciceris* from Sudan on a set of differential cultivars of chickpea. Symptoms appeared 2 weeks after inoculation as yellowing and drying of the lower leaves, dropping of petioles and rachis, improper branching, withering, browning of vascular bundles, and finally wilting of plants but no unilateral wilt was seen.He

found all the strains of*Foc* at Hudeiba, Shendi, Wadhamid, and Hawata belonged to the same race, namely race 2, whereas the new race was only found in Shambat area (Kurmut, 2002).

Determination of races in this pathogen is conceptually simple but costly in time, facilities and resources (Jiménez-Díaz *et al.*, 1993a; Jiménez-Díaz *et al.*, 1991 and Landa *et al.*,2006). Pathogenicity procedure by using differential cultivars requires at least 40 days for the analysis, and reactions can be influenced by environmental parameters (Haware and Nene,1982). There are also several sets of cultivars available and some of the differentiation is based on intermediate reactions (Sharma *et al.*, 2005). Furthermore, the disease reaction in chickpea genotypes during biological pathotyping assays can be influenced by several factors, including soil moisture, inoculum density of the pathogen and temperature(Gupta *et al.*, 1987; Landa *et al.*, 2001;Navas-Cortés*et al.*, 2007). Consequently, lack of correct adjustment for these sources of variability may give rise to misleading identification of races of *F. oxysporum* f. sp. *ciceris* or resistance assessment in chickpea genotypes.

Pathogenicity data alone provide no information about genetic diversity within or relatedness among races of the pathogen. Knowledge of genetic diversity is needed for resistance deployment to be effective and to identify shifts in race or population structure that might occur (McDonald, 1997).

Therefore, there is a need for new, consistent, improved methods for the rapid, reliable, and reproducible identification and quantification of *F. oxysporum* f. sp. *ciceris* population diversity.

2.5. Molecular genotyping of Fusarium wilt pathogen

Basic studies on the crop are limited. Genetics of the crop is not well understood. Efforts to investigate variability through molecular markers and to develop a genome map have recently been initiated. Molecular markers have proven to be powerful tools for the characterization and identification of several plant pathogenic fungi. With the advent of polymerase chain reaction (PCR), inexpensive DNA sequencing and a relatively large databank of ribosomal DNA sequences, it is now possible to more objectively characterize and identify fungal species and other pathogens (Elzein*et al.*,2008).

Rapid and reliable detection and identification of potential plant pathogens is required for taking appropriate and timely disease management measures. For many microbial species of which all strains generally are plant pathogens on a known host range, this has become quite straightforward. However, for some fungal species this is quite a challenge. One of these is F. *oxysporum*, which, as a species, has a very broad host range, while individual strains are usually highly host-specific (Lievens*et al.*, 2008). Identification of *Fusarium* spp. by morphological characters like size, shape of conidia and pigmentation are highly variable as all these characters are influenced by nutritional composition of the medium and cultural conditions(Datta *et al.*, 2011). Moreover, many strains of this fungus are non-pathogenic soil inhabitants. Thus, with regard to effective disease management, identification below the species level is highly desirable. So far, the genetic basis of host specificity in *F. oxysporum*is poorly understood. Furthermore, strains that infect a particular plant species are not necessarily more closely related to each other than to strains that infect other hosts. Despite these difficulties, recently an increasing number of studies have reported the successful development of molecular

markers to discriminate *F. oxysporum* strains below the species level(O'Donnell, 2000).

DNA-based techniques have increasingly become the tool of choice for understanding the genetic diversity and also the evolutionary history andrelationships of *Fusarium* spp especially *F. oxysporum* f. sp. *ciceris* races because it is genetic, unaffected by environmental parameters, precise and safe time. Numerous workers have already worked on molecular variation in *Fusarium* spp. (O'Donnell, 2000).

Polymerase chain reaction (PCR) has been widely and successfully employed for the identification of important plant-pathogenic fungi,(Henson and French, 1993; Martin *et al.*, 2000). In many cases, primers for these uses were based on DNA sequence polymorphisms existing within highly conserved regions of the nuclear ribosomal DNA, such as the internal transcribed spacer or the intergenic spacer region (Ward, 1994.). Although this strategy proved successful for species identification (Ward, 1994), the above regions do not normally reveal sufficient polymorphism for distinguishing subspecific groups such as formae speciales or pathogenic races. An alternative strategy in the development of markers for intraspecific taxa is based on the isolation and sequencing of distinct fragments of random amplified polymorphic DNA (RAPD), and the use of these sequences to design PCR primers that specifically amplify selected markers. This approach of using sequence characterized amplified regions (SCARs) was first applied by Paran and Michelmore (1993), and since then has been very effective for the intraspecific identification of a diversity of plant pathogens (Kelly *et al.*, 1998; McDermott *et al.*, 1994; Jiménez-Gasco and Jiménez-Diaz, 2003).

Random amplified polymorphic DNA analysis has been applied widely in the detection and genetic characterization of phytopathogenic

fungi (Williams *et al*., 1990; Brown, 1998; Miller, 1996), including race differentiation in several *formae speciales* of *F*. *oxysporum* f. sp. *Cubense* (Bentley *et al.,* 1995). Random amplified polymorphic DNA applied to the carnation wilt pathogen *F*. *oxysporum* f. sp. *dianthi* had helped researchers to identify specific band patterns that were subsequently used as probes to distinguish races of the pathogen (Manulis *et al*., 1994;Grajal-Mart´ın *et al*., 1993;Assigbetse *et al*., 1994). In previous studies, RAPD analysis was used with primers based on either known ribosomal DNA sequences or sequencing primers to characterize and differentiate the yellowing and wilt pathotypes in *F*. *oxysporum* f. sp. *ciceris*, without achieving satisfactory identification of pathogenic races (Kelly *et al*., 1994). Those results proved useful for in-plantaand in-soil detection of the wilt inducing pathotype (Kelly *et al*., 1998; Garc´ıa-Pedrajas *et al*., 1999;Jiménez-Gasco*et al*., 2001).

Simple sequence repeat (SSR) markers amplify small fragments of DNA and provides a powerful tool for taxonomic and population genetic studies (Britz *et al*., 2002). Alleles vary according to the number of repeat units present but other mutations have also been shown to be responsible for allele length variation in SSR analysis (Burgess *et al*., 2001; Slippers *et al*., 2004). The genetic variation of some Turkish isolates were studied by Bayraktar *et al.* (2008) by using RAPD and ISSR primers and the datasets provided a substantially similar discrimination among Turkish isolates and divided them into three major groups.

Races 0, 1B, 1C, 5 and 6 of *Fusarium oxysporum* f sp *ciceris*(*Foc*)were distinguished by randomly amplified polymorphic DNA (RAPD) fingerprinting. Jim´enez-Gasco*et al.* (2001) characterized *Foc* races 0, 1B/C, 5, and 6 by the RAPD primers OPI-09, OPI-18, OPF-06, OPF-10, and OPF-12 which generated RAPD marker bands. Specific primers and polymerase chain reaction (PCR) assays that identify

Fusarium oxysporum f. sp. *ciceris* and each of the *F. oxysporum* f. sp. *ciceris* pathogenic races 0, 1A, 5, and 6 were developed. *Fusarium.oxysporum*f. sp. *ciceris* and race-specific random amplified polymorphic DNA (RAPD) markers identified in a previous study were cloned and sequenced, and sequence characterized amplified region (SCAR) primers for specific PCR developed. The specific primer pairs amplified a single 1,503-bp product from all *Foc* isolates; and single 900bp and 1,000-bp products were selectively amplified from race 0 and race 6 isolates, respectively while a race 5 specific identification assays has been developed with touchdown PCR procedure. A joint use of race 0- and race 6-specific SCAR primers in a singlePCR reaction together with a PCR assay using race 6-specific primer pair, correctly identified race 1A isolates for which no RAPD marker had been found previously, (Jime´nez-Gasco and Jime´nez-Diaz, 2003).

Genetic variations among the Turkish isolates of *Foc* were analyzed using RAPD and ISSRs molecular markers. Datasets provided a substantially similar discrimination among Turkish isolates and divided them into three major groups. Thesemethods revealed a considerable genetic variation among Turkish isolates, but no correlation with regard to the clustering of isolates from different geographic regions. Analysis of molecular variance confirmed that most genetic variability resulted from the differences among isolates within regions (Bayraktar *et al.*, 2008). Gurjar *et al* (2009),demonstrated the synergistic use of gene-specific markers, ITS-RFLP, ISSR and AFLP for distinguishing Indian *Foc*isolates.

A set of isolates, representing different geographic regions of India, was used for molecular characterization with four different molecular markers, namely random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) and inter simple sequence repeats (ISSR). All the four sets of markers gave 100% polymorphism(Dubey *et al.*, 2012). The

study grouped the isolates into eight categories at genetic similarities ranging from 37 to 40%. The molecular groups partially corresponded to the chickpea-growing region of the isolates as well as races of the pathogen characterized in this study. The majority of southern, northern and central Indian populations representing specific races of the pathogen were grouped separately into distinct clusters along with some other isolates, indicating the existence of variability in population predominated by a single race of the pathogen (Dubey *et al*., 2012**)**.

CHAPTER THREE
3. MATERIALS AND METHODS

3.1. Disease survey

Thesurvey was conducted inthe major chickpea growing areas ofSudan during 2010/2011and 2011/2012 cropping seasons. The surveyed areas included Northern State, River Nile State, Khartoum State and Gezira State, whereas samples were only collectedfromNew Halfa in KassalaState and Sennar in Sennar State. Samples showing typical Fusarium wilt symptoms were collected from chickpea growing areas of the different states as shown in Fig.6 and Table 2.

The Northern Statewith Clay loam, sandy loam and sandy claysoils and the weather is very dry.The temperaturein this state reachs a maximum of 43˚C and minimum of4˚C during winter nights and the rains are very rare. River Nile State with latitudes 16-22 north and longitude 32-35 east with sandy clay soils and annual average higher temperatures of about 37.5˚C and lower of about 21˚C with rare rains (Dongola Research Station data, personal communication). It covers the area along the Nile bank. Khartoum state at the central parts of the country with clay soils, the average higher temperatures is37˚Cand the average lower temperature is 22˚C. The rains fall mostly between the months of June and September, with the mean monthly precipitation of about 14mm during the rainy season (Shambat Research Station data and khartoum.climatemps.com). It covers the central region to the south of northern states. Gezira state with heavy clay soils andaverage maximum and minimum temperatureare 30˚C and 13˚C, respectively during winter season. The average rainfalloccurs mostly between the months of May and October with a mean monthly precipitation of about 26 mm (Gezira Research Station Meteorological

data, personal communication). It covers the central parts of the country between the Blue and White Niles. Sennar State is between Latitude 13.33 N and longitude 033.37 E. The average maximum temperature is 37˚C and minimum of about 20˚C and mean monthly precipitation of about 35mm (Sennar Research Station data, personal communication). The soils are heavy clay black soils. Kassala State lies to the east of Gezira state with average maximum temperaturesof38C˚ and minimum of about 21 C˚ and mean monthly precipitation of about 20 mm.

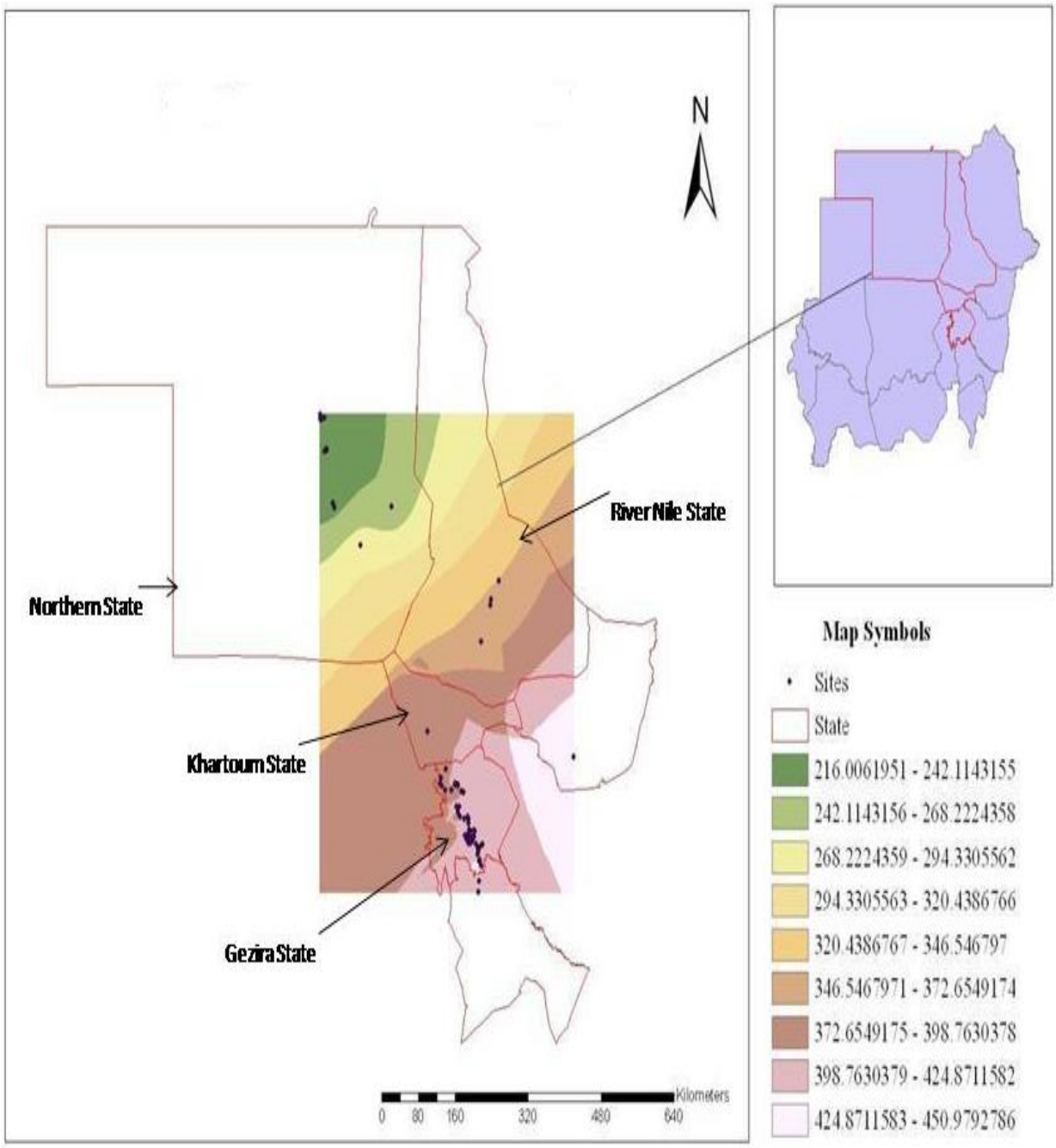


Fig. 6. Surveyed areas in Sudan for chickpea Fusarium wiltduring season 2011-2012.
Colors= Altitudes (m)

3.2 Survey methodology and data collection

Surveyed locations were chosen every 20±2 km andGIS data (longitude, latitude and altitude) were recorded.Depending on the presence of chickpea crop at each location,1-3fields were sampled. In each site, a questionnaire(appendix) was filled. The soil types of the selected sites were obtained from Land and Water Resources Research Center, Agricultural Research Corporation, Sudan. During the survey,soil type, variety sown, crops preceding chickpea in the rotation, sowing date, seeding rate, fertilizers applied to chickpea, watering intervals, wilt incidence and air temperatures during the growing seasonwere recorded. Disease incidence of chickpea wilt was recorded in each of the selected fields.

3.3. Collection of samples

A total of 170samples of wilted chickpea plants showing typical disease symptoms were collected from different locations in the six states, but only 82 isolates were recovered and used in this study. In each field, 2-3 random areas of $1m^2$each were chosen depending on field area. Disease incidence was recorded by calculating the percentage of wilted plants from the total number of plants in these areas. Samples of wilted chickpea plants were collected, labeled and placed carefully in paper bags. Seven isolates from ICARDA sick plot, Syria and Terbol, Lebanon were collected from each for comparison.Details of collected samples are shown in Table 2.

Table 2. Locations of the wilted chickpea plants samples collected in Sudan, Syria and Lebanon

State	Location	No. of samples
Northern	Dungula	3
	Artigasha	3
	Karma	3
	Alburgaig	3
	Algolid bahri	3
	Alafad	3
	Albushariya	3
	Marawi	3
Khartoum	Shambat	3
Nile River	Alalyab	3
	Hudeiba RS	4
	Barbar	3
	Shendi	3
Gezira (north)	Aldibebah	3
	Riweena	3
	Almielig	3
Gezira (middle)	*Alkabur	1
	*Alkumur	2
	Almasalamiyah	6
	Mahala and Albasatna	6
	Shlaohakhwlda	3
	Wad alnaim	3
	*Wad Medani GH	4
	Almadina Arab	3
	Haj elnour	3
	Portubail	4
	*Altalbab	1
	Altalhah	3
	Dar Essalam	3
	*El irayek	1
	*Hilat Farah	6
	Mahala	3
	*Wad Medani GRS	2
	*Um Jeer	2
	*Um Tarfaya	2
	*Wad Elabyadani&*Wad Haggar	4
Gezira (south)	Umtrebat	3
	Wad alnaw	3
	Wad alataya	3
	Bagadi	3
	Wad alhadad	2
	Alhajabdalla	2
Sennar	*Sennar	4
Kassala	*New Halfa	25
Syrian isolates	*ICARDA sick plot	7
Lebanon	*Terbol	7

* = Samples were takenfrom these locations without filling the questionnaire form.GH= Glasshouse Agricultural Research Corporation. GRS= Gezira Research Farm

3.4. Isolation, purification and identification of isolates

Infected chickpea roots and stems (5-7 cm above collar region) showing distinct vascular discoloration were washed under running water and cut into small pieces (3-5 mm). The pieces were surface sterilized with 1% aqueous solution of sodium hypochlorite for one minute, rinsed in sterilized distilled water, blotted on filter papers and plated on Potato Dextrose Agar (PDA) in 9 cm Petridishes. The plates were incubatedat 25±2 ˚Cfor 5 days.Fusarium cultures were purified by single spore cultures according to Booth (1977). Conidia mass were transferred to 10 ml sterilized distilled water and diluted 10 times. Conidial suspension of I ml was distributed on 9-cm water agar Petri-dish and incubated for 2 days at 25˚C. After incubation, each plate was inspected under a microscope and with a marking pen the germinating spores were marked. With sterilized 1mm cork borer the germinating spores were taken, cultured on PDA plates and incubated at 25±2 ˚C for7 days. The isolates were identified using the method described by Leslieand Summerell(2006) on the bases of their morphological characters.

Pure cultures of the isolates were maintained on PDAslopes in MacCartney bottles and stored at 4˚C in a refrigerator for further use.

3.5 Variability among *Fusarium oxysporum* f. sp. *ciceris* isolates

3.5.1. Pathogenic variability

Due to unavailability of enough seeds of chickpea differential lines only 25 isolates of *Foc*collected from five states in the country were chosen and tested. These isolates include13 isolates from Gezira State,2 isolates from Shambat,Khartoum State, 2 isolates from Sennar State, 5 isolates from New Halfa,Kassala Stateand 3isolatesfromHudeiba,River NileState.Disease reaction of these isolates on ninechickpea differentials,

JG-62, BG-212, C-104, JG-74, CPS-1, WR-315, Annigeri, Chafa, L550 and a susceptible Sudanese cutivar "Shendi"were studied and compared.

Inoculafrom pure single spore cultures weremultipliedon PDA media. With a 3-mm cork borer, 2 agar plugs were used to inculate 180g Sorghum Sand Mixture (SSM =135g sorghum + 45g sand + 100ml water) autoclaved at 121˚C for 20 minsin 500-ml fasks (Suliman, 1998; Ahmad *et al.*,2010).The inoculated flasks were incubated at 25˚Cand a 12-h photoperiod using fluorescent lamps for two weeks. The infested sorghum sand mixture was mixed thoroughly with4 kg sterilized soil (clay loam/sand, 1: 1, w/w). Five seeds of each differential line were surface-disinfested with 5% sodium hypochlorite before planting and sown into 20 cm diameter clay pots filled withthe infested soil mixture. The infested pots were watered and left for four days for the fungus to be established before sowing. Control plants were similarly grownin anautoclaved non-infested sorghum sand mixture and autoclaved soil. The pots were arranged in a complete randomized design with 2 replicates for each isolate differential line combination and were kept under natural conditions in the green house. The emerged plantswere observed daily for symptom development. Disease reactions were assessed and scored about 6 weeks after inoculation according to the disease rating scale ofIqbal *et al*. (1993), with some modifications where Highly Resistant (HR) = 0-10% wiltedplants, Resistant (R)= 11-20% wilted plants, Moderately Resistant (MR) = 21-40% wilted plants, Susceptible (S) = 41-80% wilted plants and Highly Susceptible (HS) ≥ 80% wilted plants. Wilted plants were checked for vascular discoloration symptoms to confirm that the disease is caused by *Fusarium oxysporum* f. sp.*ciceris*.

3.5.2. Cultural and morphological variability

The Sudanese, Syrian and Lebanese isolates of *Foc*were subjected to detailed morphological and cultural characteristics, colony appearance, colony colour, colony size on Potato Dextrose Agar medium (PDA). The cultures of all isolates were identified under the light microscope according to macro and micro conidia characteristics, chlamydospores and phialides branching on Spezieller Nährstoffarmer Agar(SNA) 1 g KH_2PO_4 1g KNO_3 + 0.5g $MgSO_4 \cdot 7H_2O$ +0.5 g KCl + 0.2 g Glucose + 0.2 g Sucrose in 1L Distilled H_2Ousing the Genus Fusarium key (Leslieand Summerell, 2006; Gerlach*et al.*, 1982).

3.5.2.1 Differences in colony diameter texture and color

The inoculated PDA plateswith isolates of *F. oxysporum* f. sp *ciceris*were incubated at 25±2˚C. Colony colour and texture were observed and colony diameter was measured after 7 days by taking four measurements at right angle from each plate. The data from the replicated plates wereaveraged.

3.5.2.2 Differences in conidiasize

Minimum and maximum length and widthof the microconidia and macroconidia for all isolatesweremeasured in micrometer (µm) using ocular micrometer. A mean of 10 observations for each isolate were recorded.

The data for colony diameter and conidia sizewere analyzed statistically with MSTATC program to check thedifferences among isolates.

3.5.3 Molecular variability

3.5.3.1 Fungal isolates preparation

Pure cultures of*Fusarium oxysporum* f sp *ciceris* isolatesmaintained on PDA and kept at 4˚C were used. Details of the isolates used are presented in Table 3. The cultures were grown on PDA plates at 25±2˚C for 7 days.With the use ofthree mm cork borer, 3-4 discs of the mycelium of each isolate were aseptically transferred to 100 ml Malt Yeast Extract (MYE) broth(6g malt extract+6g yeast extract+6g saccharose in 1L distilled water) in Erlenmeyer flasks. The flasks were incubatedin a shaker at 50 rpm at room temperature for 7 days (Fig 7).

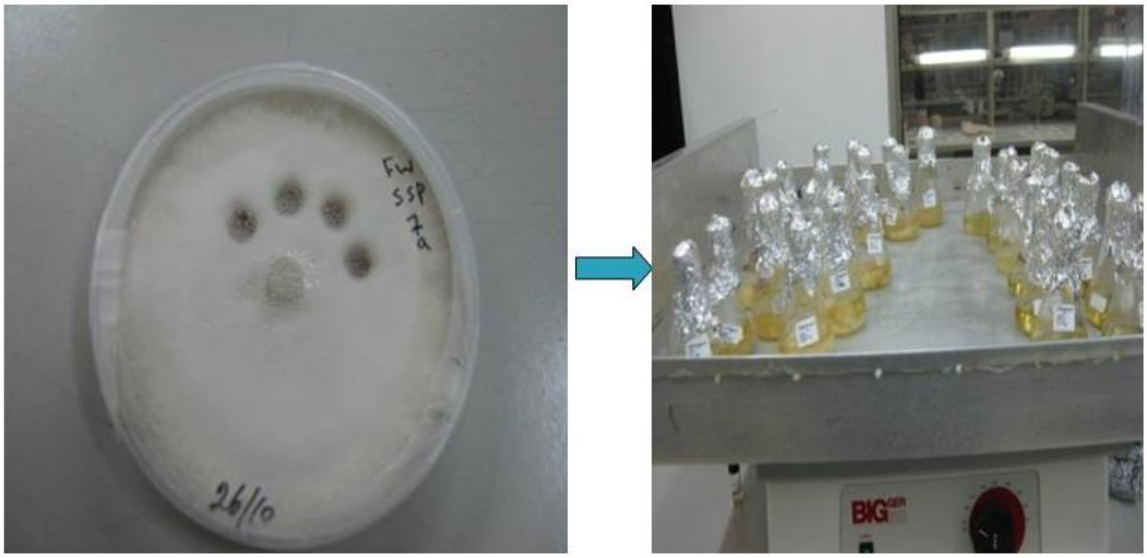


Fig. 7.*Foc* mycelium grown in MYE and incubated in a shaker

The mycelium was harvested using the method described by Raeder and Broda (1985). Cultures were filtered through a fine mesh screen to separate the mycelium whichwas then carefully covered with the mesh, tied with a plastic rubber and labeled. The labeled meshes were freeze dried at -80˚C for 3 to 4 days. The dried mycelia were ground with electric grinder into fine powderand kept in Eppendorf tubes in the fridge to be used for DNA extraction.

Table 3. Details of the isolates used for molecular diversity

Isolate	Location	State	Isolate	Location	State
Foc1	Shambat 1	Khartoum	*Foc43*	Hudeiba 1	Rive Nile
Foc2	New Halfa 1	Kassala	*Foc44*	Shambat 2	Khartoum
Foc3	Dar Essalam 1	Gezira(Center)	*Foc45*	New Halfa 20	Kassala
Foc4	Um Trebat	Gezira(South)	*Foc46*	New Halfa 21	Kassala
Foc5	NewHalfa 2	Kassala	*Foc47*	Shambat 3	Khartoum
Foc6	New Halfa 3	Kassala	*Foc48*	NewHalfa 22	Kassala
Foc7	New Halfa 4	Kassala	*Foc49*	WadHaggar	Gezira Center)
Foc8	Hilat Farah 1	Gezira(South)	*Foc50*	Portubail 3	Gezira Center)
Foc9	Wad Haggar	Gezira(South)	*Foc51*	New Halfa 23	Kassala
Foc10	Hilat Farah 2	Gezira(South)	*Foc52*	HillatFarah 5	Gezira(South)
Foc11	WdElabyadani	Gezira(Center)	*Foc53*	Hillatfarah 6	Gezira(South)
Foc12	Portubail 1	Gezira(Center)	*Foc54*	Um Jeer 2	Gezira (Center)
Foc13	New Halfa 5	Kassala	*Foc55*	Hudeiba 2	Rive Nile
Foc14	Hilat Farah 3	Gezira(South)	*Foc56*	Hudeiba 3	Rive Nile
Foc15	Alkumur 1	Gezira(Center)	*Foc57*	New Halfa 24	Kassala
Foc16	New Halfa 6	Kassala	*Foc58*	New Halfa 25	Kassala
Foc17	Sennar 1	Sennar	*Foc59*	WadMedani(GRF)1	Gezira(Center)
Foc18	New Halfa 7	Kassala	*Foc60*	Hudeiba 4	Rive Nile
Foc19	New Halfa 8	Kassala	*Foc61*	Wad Medani (GRF)2	Gezira(Center)
Foc20	New Halfa 9	Kassala	*Foc62*	Terbol 1	Lebanon
Foc21	Um Jeer 1	Gezira(Center)	*Foc63*	Bagadi 1	Gezira(South)
Foc22	New Halfa 10	Kassala	*Foc64*	Almasalamiyah1	Gezira(Center)
Foc23	New Halfa 11	Kassala	*Foc65*	Bagadi 2	Gezira(South)
Foc24	New Halfa 12	Kassala	*Foc66*	Wad Medani (GRF)3	Gezira(Center)
Foc25	Sennar 2	Sennar	*Foc67*	Altalhah	Gezira(Center)
Foc26	Portubail 2	Gezira(Center)	*Foc68*	Wad Medani (GH)1	Gezira(Center)
Foc27	Portubail 3	Gezira(Center)	*Foc69*	Wad Medani (GH)2	Gezira(Center)
Foc28	New Halfa 13	Kassala	*Foc70*	Wad Medani (GH)3	Gezira(Center)
Foc29	New Halfa 14	Kassala	*Foc71*	Terbol 2	Lebanon
Foc30	Dar Essalam 2	Gezira(Center)	*Foc72*	Wad Medani (GH)4	Gezira(Center)
Foc31	El irayek	Gezira(Center)	*Foc73*	Terbol 3	Lebanon
Foc32	Alkumur 2	Gezira(Center)	*Foc74*	Alburgaig1	Northern
Foc33	Dar Essalam 3	Gezira(Center)	*Foc75*	Alburgaig2	Northern
Foc34	Altalbab	Gezira(North)	*Foc76*	Alburgaig3	Northern
Foc35	Um Tarfaya	Gezira(Center)	*Foc77*	Alkabur1	Gezira(Center)
Foc36	New Halfa 15	Kassala	*Foc78*	Almasalamiya2	Gezira(Center)
Foc37	New Halfa 16	Kassala	*Foc79*	Almasalamiya3	Gezira(Center)
Foc38	New Halfa 17	Kassala	*Foc80*	Barbar	Rive Nile
Foc39	Hillat Farah 4	Gezira(South)	*Foc81*	Aldibebah1	Gezira(North)
Foc40	Mahala	Gezira(Center)	*Foc82*	Aldibebah2	Gezira(North)
Foc41	NewHalfa 18	Kassala	*Foc83*	Albushariyah2	Northern
Foc42	New Halfa 19	Kassala	*Foc84*	Albushariyah3	Northern

Cont- Table3. Details of the isolates used for molecular diversity

Isolate	Location	State	Isolate	Location	State
Foc85	Aldibebah3	Gezira(North)	*Foc91*	ICARDA SP2	Syria
Foc86	Terbol 4	Lebanon	*Foc92*	ICARDA SP3	Syria
Foc87	Terbol 5	Lebanon	*Foc93*	ICARDA SP4	Syria
Foc88	Terbol 6	Lebanon	*Foc94*	ICARDA SP5	Syria
Foc89	Terbol 7	Lebanon	*Foc95*	ICARDA SP6	Syria
Foc90	ICARDA SP1	Syria	*Foc96*	ICARDA SP7	Syria

GH= Glasshouse, Agricultural Research Corporation (ARC), WadMedani. GRF= Gezira Research Farm, Wad Medani. ICARDA SP= ICARDA Fusarium wilt sick plot

3.5.3.2 DNA Extraction

The DNA for each isolate was aseptically extracted using CTAB (Cetyl Tri methyl Ammonium Bromide) method(Murray and Thompson, 1980). The CTAB solution was prepared by dissolving 40.9g sodium chloride (NaCl), 3.78g of Ethylene Diamine Tetra Acetic acid (EDTA, disodium salt) , 10g of Cetyl Tri methyl Ammonium Bromide and 6.05g Tris-base[tris(hydroxymethyl) aminomethane]in 1L of distilled water and autoclaved for 15 minutes at 121˚C. One ml CTAB solution was added to 50 mg of the mycelia powder in 2 ml Eppendorf tube. The tubes were heated in a water bath at 65˚C for 1 h with converting or shaking the contents every 15 minutes to break the cell walls of the fungus. The tubes were cooledin an ice for 5 minutes and 1ml of chloroform isomyl alcohol 24:1 was added to separate the DNA from other contents of the fungal cells. The tubes were placed in a closed box and their contents were thoroughly handskaken for 20 minutes and centrifuged for 10 minutes at 10000 rpm. The upper aqueous phase, which contains the DNA pellets, was carefully transferred to a clean labeled2ml Eppendorf tube. Then 5µ of RNAase were added and the contents were shaken well to degrade the RNA. The tubes were then placedin a water bath at 37˚C for 45 minutes. 1 ml of isopropanol solution was added and the contents were left in a freezer at -20˚C for 15-30 minutes for the DNA pellets to precipitate. After

freezing, the contents were centrifuged at 14000 rpm for 5 minutes and then the supernatant was decanted and DNA pellets were collectedat the bottom of each tube. The DNA was washed twice by adding 1 ml of 70% alcohol and centrifuged for 2 minutes at 10000rpm. Then the alcohol was completely decantedfrom the tubes and the DNA was left to dry at room temperature. Finally, 60-100 ml of 0.1X TE buffer (Tris-baseEDTA) was added to the DNA and kept in the fridge at 4˚C to dissolve the pellets.

3.5.3.3. TBE (Tris-Borate EDTA) buffer preparation

To prepare 10XTBE buffer, 108g Tris-base were addedto 55g Boric acid and 7.5g EDTA.The contents were dissolved in 1L of distilled water in an electric shaker. To make 1XTBE buffer, 90ml distilled water were added to 10ml of 10XTBE.

3.5.3.4.Gel preparation

3.5.3.4.1.1% Agarose gel preparation

Agarose gel 1% was prepared by suspending 1g Agarose in100 ml 1X TBE buffer in500 ml Erlenmeyer flask. The suspension was boiled and dissolved in a microwave for 3 minutes and then cooled at room temperature. After cooling, 7-10µ of Ethidium bromide liquid were added and hand shaken for complete mixing. After leveling the tray and adjusting the combs, the warm liquid Agarose was pouredcarefully into the tray to avoid bubbles. After complete drying of the gel, it was transferred to the electrophoresis apparatus with the wells near the negative electrode and filled with TBE buffer until it was 5mm above the gel (Fig. 8 A and B).

3.5.3.4.2 1.5% Agarose gel preparation

1.5% Agarose gel was prepared by suspending 1.5g Agarosein 100ml 1X TBE bufferin 500 ml Erlenmeyer flask as in **3.5.3.4.1**

3.5.3.5 Testing the DNA quality

In a PCR plate, 2 µ DNA of each isolate were distributed and 5µ of dark violet loading buffer were added to add the color for the mixture, track DNA movement and increase density so that DNA sank to the bottom of the well when it was loaded.A micro pipettewas used to load 7 µ ofthe DNA mixture of each isolatein the submerged Agarose gel holes. The electrophoresis was carried out at 80-100V. After running the DNA to more than half of the distance between combs, it was stopped and the gel was taken for imaging. The gel was exposed to UV light in BIORAD IMAGER, photographed and saved.

3.5.3.6. DNAdilution

The concentrations of the DNA for all isolates were measured by a nano-drop spectrophotometer. The concentrations were dilutedto 50ng/µ solution with distilled water for all the isolates

3.5.3.7. PCR analysis

3.5.3.7.1.*Fusarium oxysporum* f. sp. *ciceris*-specific PCR protocol (for pathogenic *Foc*)

Primer sets (FOCP1 and FOCP2) specifically designed from a SCAR sequence for the identification of *F. oxysporum* f. sp. *ciceris*were used(Jiménez-Fernández*et al.*, 2011).Thereafter, several parameters of the PCR protocol were evaluated to optimize amplification results. Primer designation, sequences and PCR products with optimized amplification conditions were presented in Table3. The optimized reaction mixture was similar for all primers with final volume of 10 µl **(**3.3µl distilled water,

2µl PCR buffer (10 mM Tris-HCl pH 8.0), 1µl dNTPs (2mM), 0.6µl $MgCl_2$ (25mM), 1µl of each of the primer pairs (15 pmol/µl), 1µl (50 ng) of template DNA and 0.1µlTag DNA polymerase (5u/µl**)**.The mixture was distributed in a PCR plate and well covered with a PCR plate to avoid evaporation during amplification. Amplifications were performed in a (Bio-Rad T100) thermocycler (Fig.8C). The cycling program included an initial denaturation step of 1 min at 94°C, followed by 25 cycles of 30 s denaturation at 94°C, 30 s annealing at 55°C and 30 s extension at 72°C. The final cycle consisted of 5 min at 72°C which could be followed by a 4°C soak when needed.

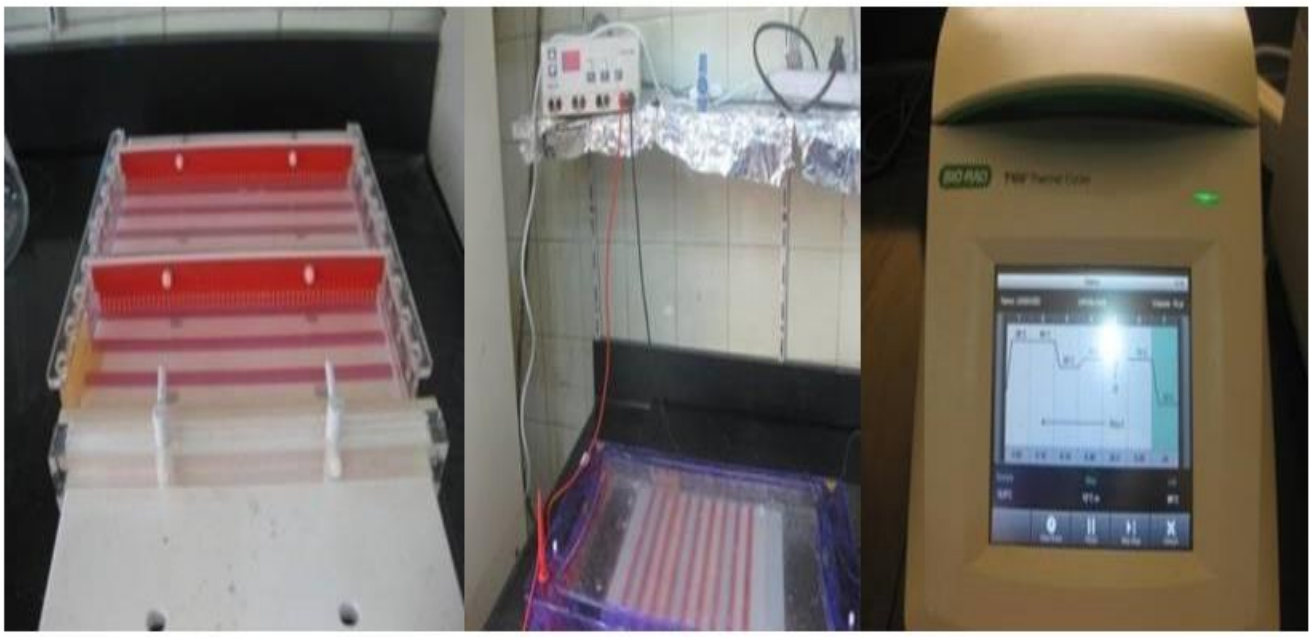


Fig.8. Gel tray balanced with two combs (A), Elctrophoresis machine (B), PCR machine (C).

3.5.3.7.2. Random amplified rolymorphic DNA(RAPD) primers

For RAPD analysis, four random primers were selected after screening of 27 RAPD primers, namely (OPF-16, OPF-12,OPF-06 and OPI-01). Amplification was performed in a 10 µl reaction volume for each isolate. The mixture containing3.3µl distilled water, 2µlPCR buffer (10 mM Tris-HCl pH 8.0), 1µl dNTPs (2mM), 0.6µl $Mgcl_2$(25mM), 1µl of RAPD primer pair (15 pmol/µl), 1µl(50 ng) of template DNA and 0.1µlTag DNA polymerase (5u/µl), was distributed in a PCR plate and well covered with a PCR plate cover to avoid evaporation during amplifications. Amplifications were performed using(BIO-RAD.T100) thermal cycler (USA),programmed for one cycle of initial denaturation at 94°C for 5 min, 40°C for 2 min and 72°C for 1.5 min followed by 35 cyclesof 94°C for 1 min, 40°C for 1.5 min and 72°C for 2 min. The amplification was completed with a 5 min final extension at 72°C.

Amplified products were loaded in 1.5% Agarose gel(prepared in 3.7.2.)and 1-kb (Fermentas)ladder was used as marker.The electrophoresis was carried out at 80-100V (Fig. 8C). After running the DNA to more than half of the distance between combs, it was stopped and the gel was taken for imaginglab.The gel was photographed under UV light by using Bio-Rad Gel-doc system with image lab software. The image was saved and documented for analysis.The experiment was repeated twice with each primer before final scoring.

3.5.3.7.3. Simple sequence repeats primers(SSRs)

The SSR markers used by Dubey *et al.* (2012) for determining variability of F. *oxysporum* f sp *ciceris*isolates were used in this study.

The SSRs primers tested against all the isolates were MB14, MB17 and MB18. The PCR reaction mixture and amplification procedure was as described previuosly for RAPD primers(**3.5.3.7.2**).The PCR reaction

mixture (10µl) consisted of 3.3µl distilled water, 2µlPCR buffer (10 mM Tris-HCl pH 8.0), 1µl dNTPs (2mM), 0.6µl $mgcl_2$(25mM), 1µl of each primer pair (15 pmol/µl), 1µl(50 ng) of template DNA and 0.1µlTag DNA polymerase (5u/µl). The mixture was distributed in a PCR plate and well covered with a PCR plate cover to avoid evaporation during amplification. The cycling program is 94°C for 5 min for initial denaturation followed by 35 cycles of denaturation at 94°C for 2 min and extension at 72°C for2 min with an elongation at 72°C for 7 min. Appropriate annealing temperatures (60.2C° for MB14, 55^0C for MB17 and 60C° for MB18) for 2 min were used for each primer set. Amplification products were resolved by electrophoresis on Agarose gel(1.5%) and 1-kb (Fermentas)ladder was used as marker. The gel wasphotographed under UV light by using Bio-Rad Gel-doc system with image lab software. The experiment was repeated twice with each primer before final scoring.

3.5.3.7.4. Race specific orsequence characterized amplified region (SCARs)

These primers were developed byJiménez-Gasco. *et al.* (2003).Amplifications were performed using(BIO-RAD.T100) thermal cycler (USA),programmed of an initial step of 2 min at 94°C, 30 cycles of 30 seconds at 94°C, 1 min of annealing temperature, 30 seconds at 72°C, followed by a final step of 4 min at 72°C for specific primer pairs of **FocR0 M15**specific for race 0, **FocR1B/CN5** specific for race1B/C,**FocR6-O2**specific for race 6and**FocR6P18f/FocR0-M15r**specific for race 1A of the pathogen (Table3).For race 5-specific primer pair(**FocR5-L10**), a touchdown-PCR procedure was used to ensure specificity of the amplification product. This procedureconsisted of 2 min at 94°C; 10 cycles at decreasing annealingtemperature (71 to 61°C); 15 cycles of 30 s at 94°C, 1 min at 61°C, and 30 s at 72°C; followed by 4 min

at 72°C. PCR amplification products were separated and visualized as described above for the RAPD reactions.

3.5.3.7.5.Gene specific marker (Hop78, Dst and Xyl) to identify race 2, 3 and 4

These markers were developed by Gurjar. *et al.*(2009).The reaction mixture for these primers is the same as for the other primers (10µl total volume). PCR conditions were initial denaturation at 94 ˚C for 5 min, followed by 25 cycles of 94 ˚C for 1 min denaturation, 50 ˚C for 30 s annealing and72 ˚C for 30 s extension. Final extension was carried out at 72 ˚C for 10 min. PCR products were viewed on 1.5% agarose gel by electrophoresis in TBE buffer. DstF/R and XylF/R primers with the same PCR reaction mixtures as Hop78 primer were used for all isolates.

The experiment was repeated twice with each primer before final scoring.

Table4. List of the primers sequences and annealing temperatures (˚c)

Primer type	Primer name	Sequence	Annealing Temp˚C
RAPD	OPI-1	ACCTGGACAC	40
	OPF-06	GGGAATTCGG	40
	OPF-12	ACGGTACCAG	40
	OPF-16	GGAGTACTGG	40
SSRs	MB14	CGTCTCTGAACCACCTTCATC TTCCTCCGTCCATCCTGAC	60.2
	MB17	ACTGATTCACCGATCCTTGG GCTGGCCTGACTTGTTATCG	55
	MB18	GGTAGGAAATGACGAAGCTGAC TGAGCACTCTAGCACTCCAAAC	60
SCARs	FocR0-M15	GGAGAGCAGGACAGCAAAGACTA GGAGAGCAGCTACCCTAGATACACC	61
	FocR1B/C-N5	GAGAGCAGGGTCAGCGTAGATAG GCAGCAGAAGAGGAAGAAAATGTA	61
	FocR5-L10	GGAAGCTTGGCATGACATAC AAGCTTGGGCACCCTCTT	71-61
	FocR6-O2	GAGCAGTCAATGGCAATGG AGAGCAGGGTCAGCGTAGATA	61
	FocR6P18f FocR0-M15r	GGAGAGCAGTAGAGTTACAGCAGTATT GGAGAGCAGCTACCCTAGATACACC	61
Specific for pathogenic *Foc*	FOCP1	TACGGTACCAGATCATGGCGT CGCTTTCGATCGTGGCTATG	55
	FOCP2	CATGGTTTCGTTAGGCCAGT CGCAGTCTTCGTCGTCATTA	55
Gene specific	HOP78F2/R2	CTTTTGGCATGAGATTGTAGCCTC CGTGGGGTTATACCTCTAGGCTA	50
Gene specific	DST F/R	ATG GTT AAA GAC ACA AAG CC GTT TGA AAC TCA GTC TCG TTG CG	50
Gene specific	XYLF/R	GAC AAY AGC ATG AAG TGG GAT ACA CCC CAD ACR GTR ATD CC	50

3.6. Data analysis

The GIS data (Longitude, Latitude and Altitude) for the locations was entered to the computer and map of the disease was done. The data recorded from the questionnaire was entered to the computer and analyzed by SPSS(**S**tatistical **P**ackage for the **S**ocial **S**ciences) 16.0 program for windows (SPSS Inc., Chicago, Illinois, U.S.A).The primers that gave reproducible and scorable amplifications were used for the analysis. The DNA bands were scored as binary digit code of "0" (for absence) and "1" (for presence), respectively. Binary matrices were analyzed by DARwin5 (Version 5.0.158). Genetic similarity between pairs was estimated using Jaccard's coefficients which were clustered to construct a dendrogram by using clustering programme through Unweighted Pair Group Method with Arithmetic Mean analysis (UPGMA)(Rohlf, 1998).

3.7. Screening of chickpea germplasm to wilt resistance

Twenty chickpea germplasms (8 cultivars + 12 lines) (Table 20) were evaluated for their reaction to races 0, 2 and X (unidentified) under greenhouse conditions. The inoculum of each of the three races was increased in SSM as described in **3.5.1** and then mixed thoroughly with autoclaved soil (clay loam/sand, 1: 1, w/w). Surface sterilized ten seeds of each germplasm were sown in a separate pot filled with infested SSM. The pots were arranged in a completely randomized block design with 2 replicates for each race-germplasm combination and were kept in the green house under natural conditions. The plants were observed daily for symptom development. Disease reactions were assessed and scoredsix weeks after inoculation. Disease reaction was graded using the rating scale of Iqbal *et al.*(1993) with some modifications where Highly Resistant (HR)=0-10% wilted plants, Resistant(R)=11-20% wilted plants, Moderately Resistant

(MR)= 21-40% wilted plants, Susceptible (S)=41-80% wilted plants and Highly Susceptible (HS)more than 80% wilted plants.

CHAPTER FOUR

4.RESULTS

4.1. Disease survey

4.1.1. Distribution of Fusarium wiltdisease in chickpea growing areas in Sudanseason 2011/2012

The results of the surveys indicated thatthe disease is prevalent in all the surveyed states despite the differences insoil types.The soil types in the Northern State locations are clay loamy, sandy clay and sandy loams, whereas for the River Nile State, it is sandy clay. Shambat location inKhartoum Statehas clay soils. In the northern parts of Gezira State the soils are light clay, whereas they are heavy clay in the central and southern parts(Table 5).

Table 5.GIS data and soil type for the samples' locations

State	Longitude (E)	Latitude (N)	Altitude (m)	Soil type*
Northern	30.4-31.81	18.48-19.62	217-249	Clay loam-sandy loam-sandy clay
River Nile	33.57-33.92	16.78-17.55	352-364	Sandy clay soils
Khartoum	32.52	15.65	284	Clay soils
Gezira	33.05- 33.60	13.78-15.18	395.428	Heavy clay soils
Sennar	33.37	13.33	418	Heavy clay soils
Kassala	35.36	15.19	451	Clay soils

***=** Thesource is **Land and Water Resources Center**, Gezira Research Station, Wad Medani

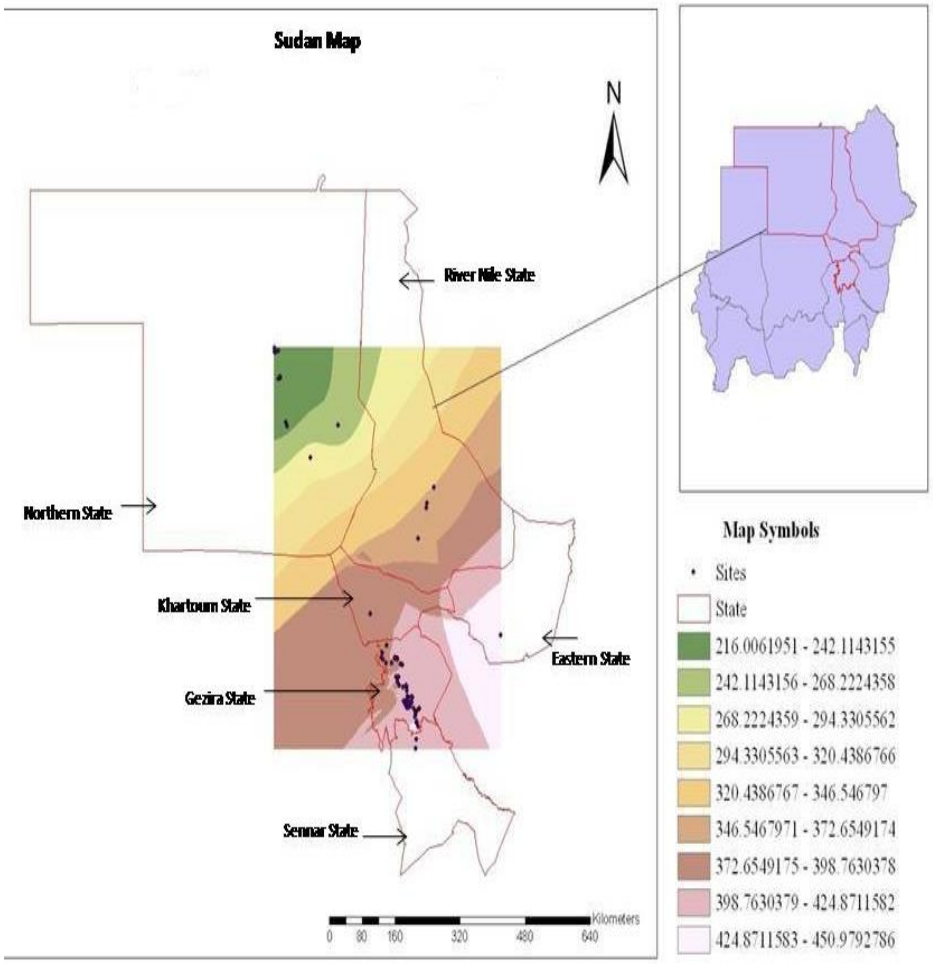


Fig.9. Chickpea growing areas from which samples were collected during seasons 2010/2012

From the survey data, 60% of the collected samples were from Gezira State. This was obviously, because the chickpea cultivated areas are very largeas compared to other states. Chickpea production retreated in the northern states especially in the River Nile State due to high incidence of chickpea wilt disease and only very few areas were cultivated with chickpea "Baladi"cultivar. The prevalence of the disease in Gezira State was very high and all samples collected were infected with chickpea wilt.

4.1.2. Fusarium wilt incidence

Incidence of chickpea wilt disease across the six surveyed states ranged from 0-100%. In the Northern State,variability in wilt incidence was very high.Some fields were free of the disease while others were 100% infected with the disease. In the southernparts of this state (Marawi and Alafad)andsome locations in the northern part of Gezira State, the incidence of the disease did not exceed 5% (Table 6).

From the average wilt incidence in the surveyed states south of Gezira had the highest disease incidence (27%), followed by the Northern State (21%), central Gezira (21%) and Khartoum State (20%). The River Nile State and the northern parts of Gezira State showed the lowest wilt incidences of 12 and 11, respectively (Table 6 and Fig. 10).

Table 6. Wilt incidence (%) for the surveyed locations and states in Sudan season 2011/2012

State	Location	Soil type	No of fields	Incidence/ location	mean wiltincidence/ location	Mean wilt incidence/ State
Northern	Dungula1	Clay loam	1	0	0	21
	Dungula2	Clay loam	1	100	100	
	Artidi	Sandy loam	2	0	0	
	Artigasha	Sandy loam	1	10	10	
	Karma Wadikhalil	Sandy clay loam	1	5	5	
	Alburgeg	Sandy loam	2	0-100	50	
	Algolid(Bahri)	Clay loam	1	40	40	
	Algolid(Gibli)	Clay loam	1	0	0	
	Albushariya	Sandy clay	1	20	20	
	Marawi	Sandy clay	1	5	5	
	Alafad	Clay loam	1	5	5	
Khartoum	Shambat	Clay	1	20	20	20
	Shambat	Clay	1	20	20	
River Nile	Alalyab North	Sandy clay	2	5-5	5	12
	Alalyab South	Sandy clay	1	1	1	
	Hudeiba	Sandy clay	3	0-40	20	
	Shendi	Sandy clay	1	20	20	
North Gezira	Aldibeba1	Light clay	1	20	20	11
	Aldibeba2	Light clay	1	20	20	
	Aldibeba3	Light clay	1	5	5	
	Riweena	Light clay	2	0-10	5	
	Almielig	Light clay	2	5-5	5	
Central Gezira	Almasalamya1	Heavy clay	2	5-5	5	21
	Almasalamya2	Heavy clay	2	5-5	5	
	Almasalamya3	Heavy clay	2	0-40	40	
	Mahala	Heavy clay	2	0-40	40	
	Albasatna	Heavy clay	2	20-20	20	
	Shlaohakhwlda	Heavy clay	2	20-20	20	
	Wad alnaim	Heavy clay	2	10-10	10	
	Purtubail	Heavy clay	2	30-30	30	
	Dar Essalam	Heavy clay	2	0-40	40	
	Almadina Arab	Heavy clay	2	0-20	10	
	Haj elnour1	Heavy clay	2	5-5	5	
	Haj elnour2	Heavy clay	2	20-40	30	
South Gezira	Bagadi	Heavy clay	2	20-20	20	27
	Wad alnaw	Heavy clay	3	0-60	30	
	Wad alataya	Heavy clay	3	30-60	40	
	Umtrebat	Heavy clay	3	10-70	30	
	Umtrebat west	Heavy clay	3	20-50	40	
	Wad alhadad1	Heavy clay	2	40-40	40	
	Wad alhadad2	Heavy clay	2	5-5	5	
	Alhajabdalla1	Heavy clay	2	0-30	15	
	Alhajabdalla2	Heavy clay	2	20-20	20	

There were no differences in disease incidence in the sandy and clay loam soils of the Northern State and the clay soils of Khartoum, but there were differences between central, southern and northern parts of Gezira State. In Sandy clay soils of the River Nile State and light clay soils of northern Gezira State, wiltdisease incidence is lower than in all other soil types (Fig 10).The disease was most prevalent in Gezira State with mean disease incidence of 27, 21 and 11% in south, central and north Gezira, respectively. The respective mean disease incidence in the Northern, Khartoum and River Nile States was 21%, 20% and 12% (Fig. 10).

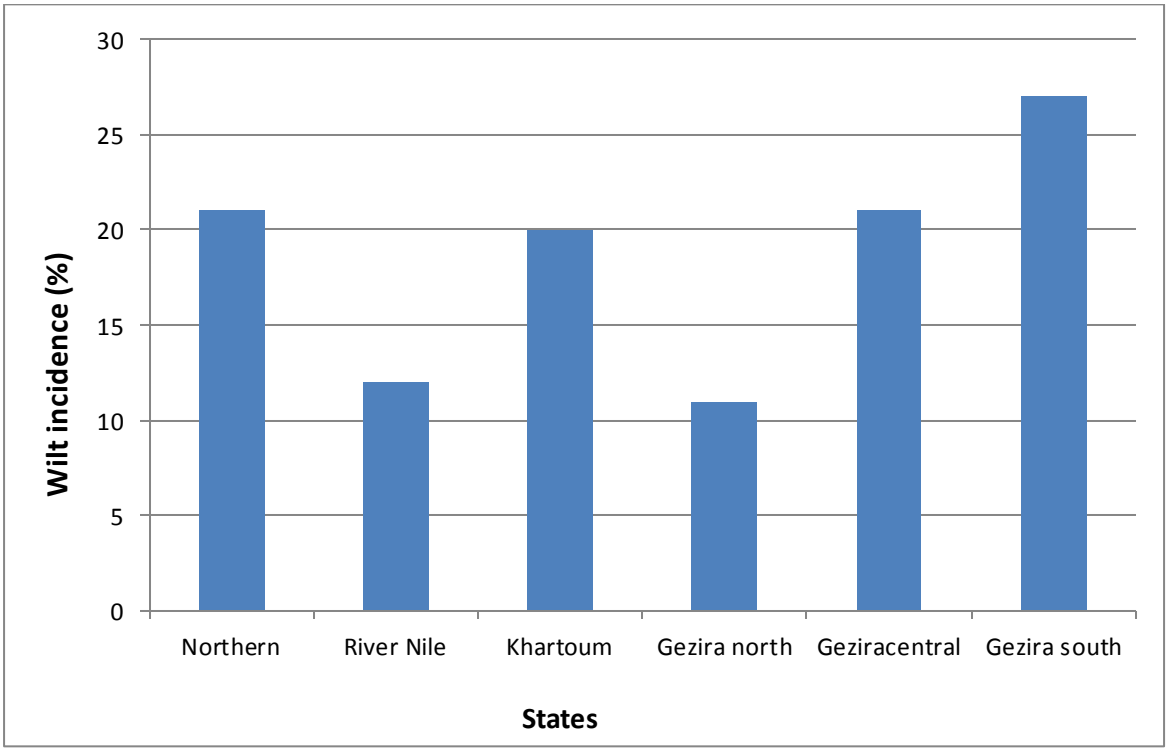


Fig. 10. Chickpea wilt incidence in different states in Sudan during season 2011/2012

4.1.3. Relationship between chickpeawilt incidence andcultivars grown

In Sudan, chickpea was traditionally grown in the northern stateswhere farmers used to grow the local"Baladi"cultivar. During the last two decades chickpea growing areas were extended to the central parts or states of the country in areas of about 15000 ha.From the results of the survey about 74% of the total interviewed farmers weregrowning"Baladi" cultivar which occupied about 64% of the total surveyed area.The released chickpea cultivars,Atmour, Wad hamid,Shendi, Jebel Marraand others which are high yielding and known to be more resistant to wilt and root rots and viral diseases occupied about 30,2,0.02, 0.02 and3% of thetotal area , respectively(Fig 11). The Baladi cv.was obtainedfrom the local markets andsometimes from the previous season harvest that had been in traditional farmers'stores.

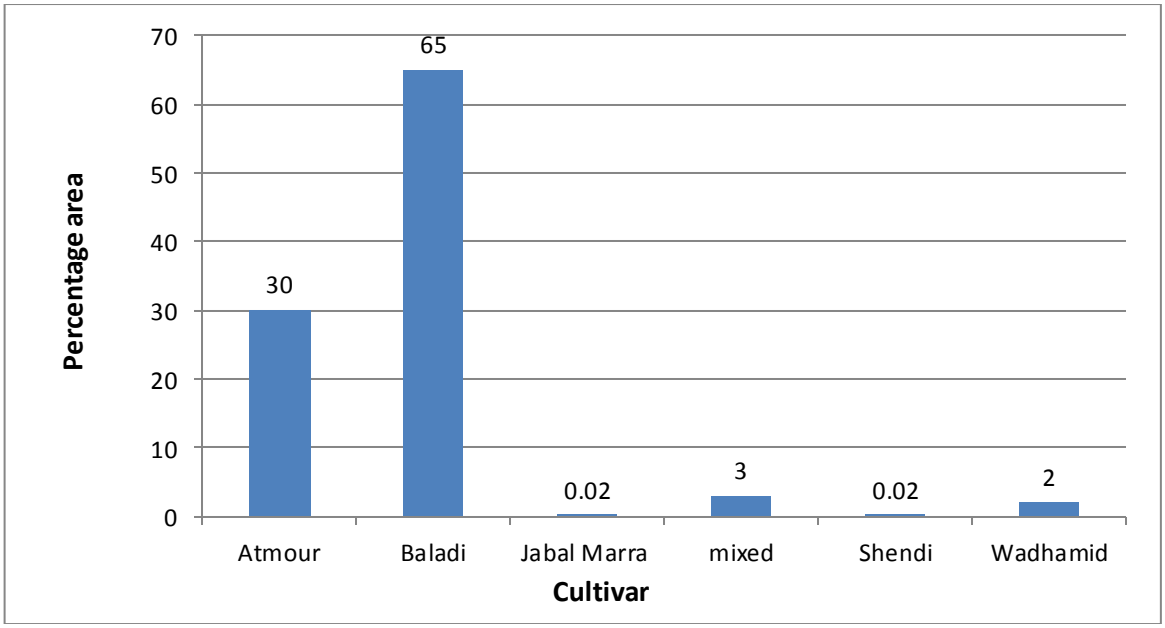


Fig.11. Chickpea cultivars grown and their areas during season 2011/2012

The mean incidence disease of the wiltin the grown cultivars ranged from 10 to 25% (Fig.12). The mixed cultivars exhibited 25% disease incidence.The cultivars "Baladi", Shendi and Jebel Marra displayed high disease incidenceof about 20%. Wad Hamid cultivar showed a mean disease incidence of 12%, while the least susceptible cultivar Atmour showed 10% disease incidence (Fig. 12).

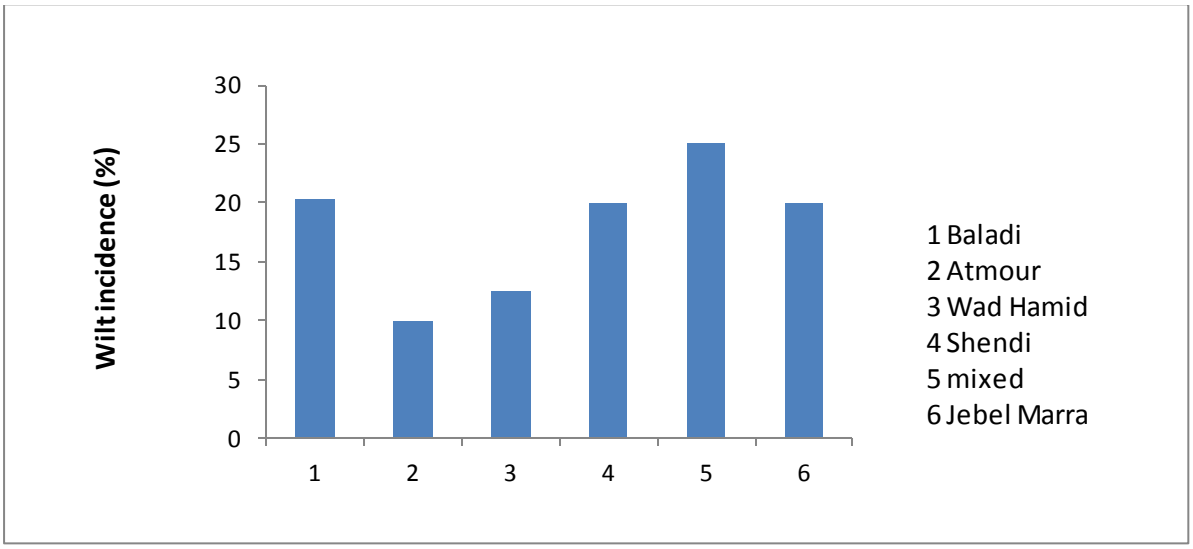


Fig. 12. Chickpea wiltincidence ofthe cultivated varieties during season 2011/2012

4.1.4. Relationship between chickpea wilt incidence and the preceding crops in the rotation

The results of the farmer's questionnaire clearly indicated that chickpea was grown in the different surveyed states and waspreceded by a variety of crops including cereals, cotton, groundnut, several vegetablesand other crops. The area under chickpea following cereals in the rotation accounted for 40% of the total surveyed area. Fallow system preceded chickpea accounted for15% of the surveyed area followed by lower areas of 8% for maize and 5% for each of onion, peanutsand monocroping. Generally, there was no significant differenceamong percentage areas of chickpea grown after different crops.Disease incidence was highest inchickpea monocroing system which accounted for 42%.The incidence of the disease in chickpea crop preceded by cereals, cotton, fallow and vegetables accounted for 22, 20, 12 and 8%, respectively (Fig.13)

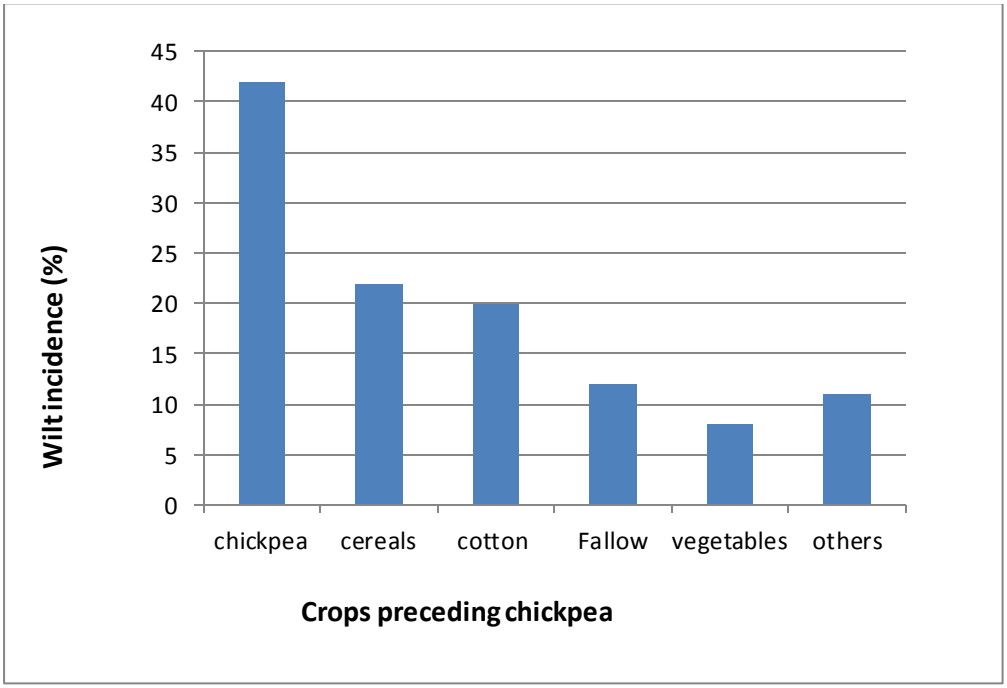


Fig. 13. Crops preceding chickpea in the rotation and wilt incidence during season 2011/2012

Table 7. Crops preceding chickpea in each state in Sudan during season 2011/2012

Northern State	River Nile State	Khartoum State	Gezira North	Gezira center	Gezira south
Fallow chickpea Egg plant Maize Sugar beet	Sorghum Okra Wheat Alfa Alfa	Wheat chickpea	Peanut Sorghum watermelon	Sweetpotato sorghum carrots Pigeon pea Chickpea onion	Fallow cotton Sorghum

4.1.5. Relationship between chickpea wilt incidenceandsowing date

There were variations in sowing dates of chickpea among the interviewed farmers. About 20% of the interviewed chickpea farmers followed sowing dateson mid November whichwas recommended by ARC to obtain higher yields. About 40% of the farmers grow chickpea on early November to obtain early harvest before other farmers to catch the highest market prices. About 40% of the farmers strongly believe in late sowing on late November to late December to escape high temperature at the beginning of winter and consequently wilt and Chlorotic stunt virus diseases.

The results of this study indicated a relationship betweenincidence of wilt disease of chickpea and sowing date. In general, disease incidence declined with late sowings. Early November sowing showed the highest disease incidence (31%),followed by mid November (19%), late November (18%) and early December sowings(17%) (Table 8). Sowingin mid to late December considerably reduced disease incidence to only 5%.The incidence of wilt disease in the three November sowing dates was similar.

The results clearly indicated a strong correlation between disease incidence and sowing dates (Fig. 14)

Table 8. Chickpeawilt incidencein relation to sowing date

sowing dates	Wilt incidence (%)
1-10 November	31
11-20 November	19
21-30 November	18
1-10 December	17
11-25 December	5

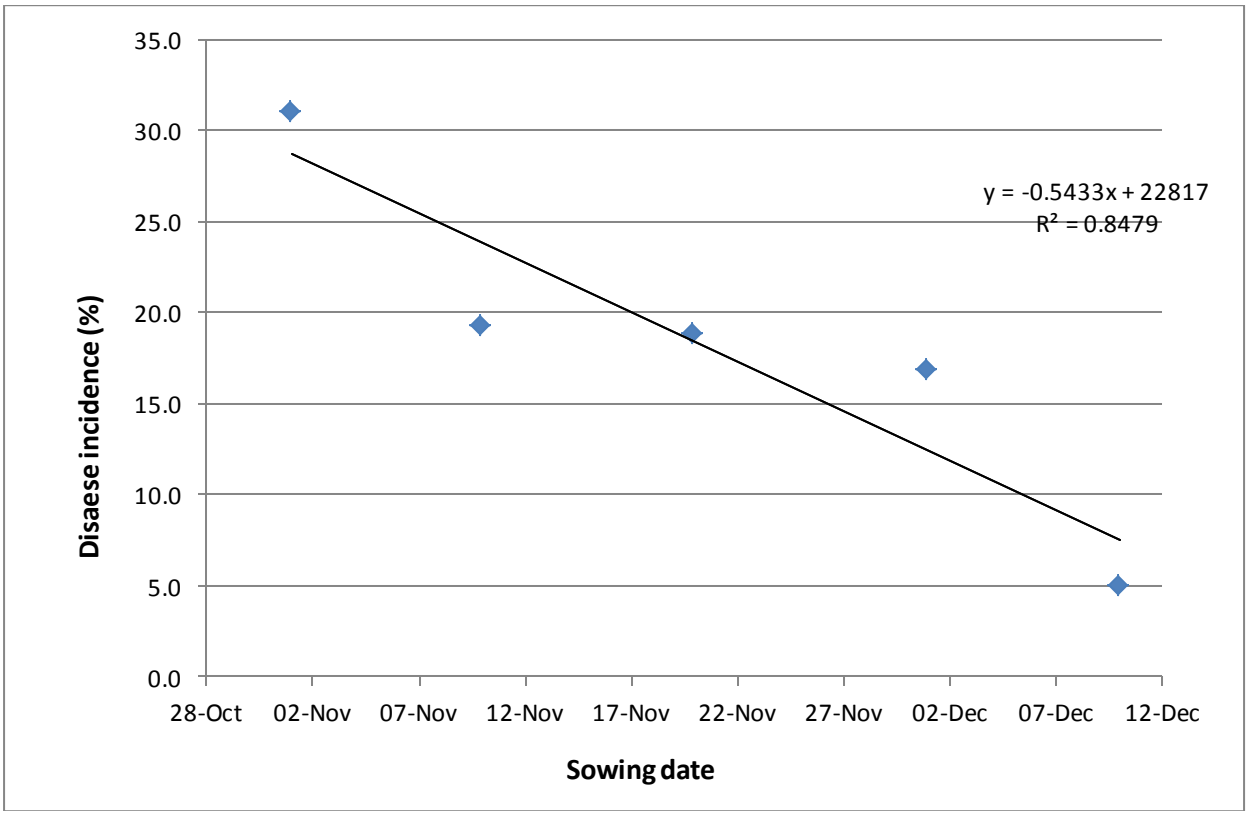


Fig. 14. Relationshipbetweenchickpea wilt incidenceand sowing date

4.1.6. Relationship between chickpeawilt incidenceand seeding rate

The results showed that only 8% of the interviewed farmers used the seed rate recommended by ARC (60 kg/ha). The majority of the farmers (80%)used seed ratesof 15-57kg/ha. Only 20% of the farmers used a seed rate of62-125kg/ha.

Erratic disease incidence (12.5-27.5%) was observed in farmers fields using seed rates of 10-80kg/ha and there was no consistent relationship between wilt incidence and seeding rate (Table 9 and Fig. 15). The highest disease incidence (50%) was recorded when farmers applied higher ratesof more than 80kg of chickpea seeds/ha. The correlation is only prominant when seed rate was over 80kg/ha (Fig. 15).

Table 9.Chickpea wilt incidence in relation to seeding rate

seeding rates (kg/ha)	wilt incidence(%)
10-20	14.2
21-30	12.5
31-40	17.2
41-50	27.5
51-60	23.1
61-70	14.0
71-80	25.0
≥ 80	50.0

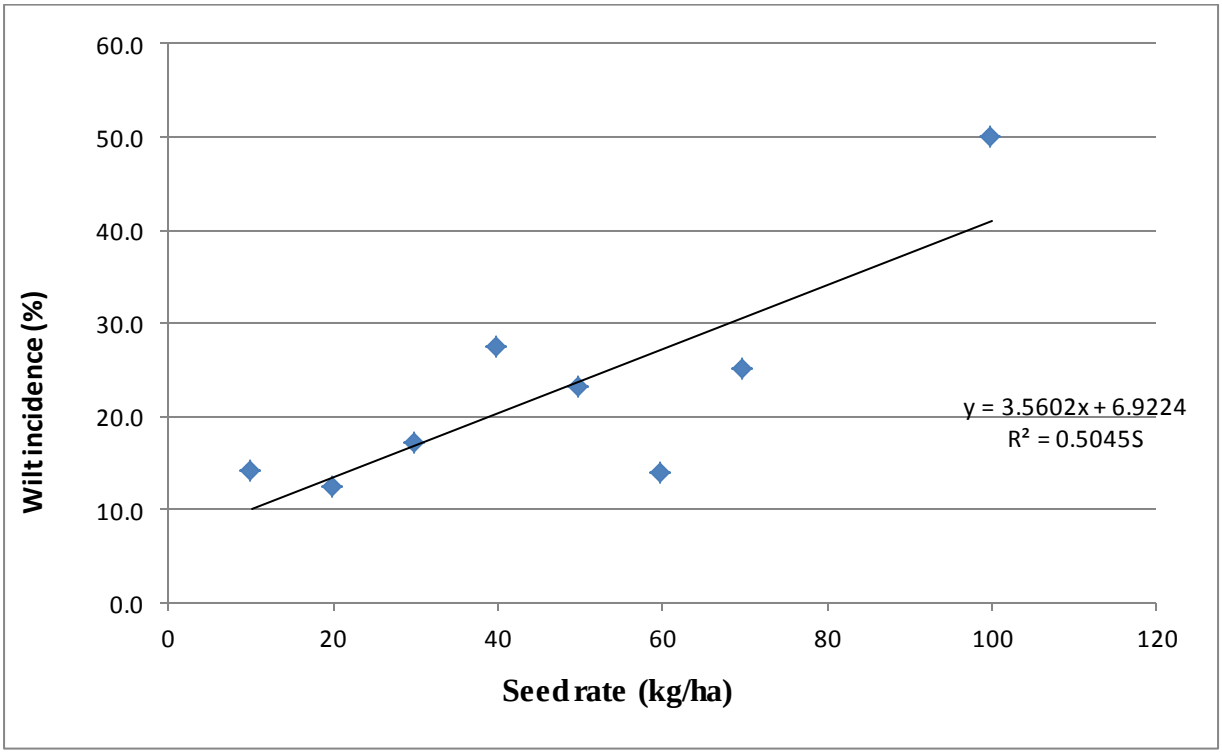
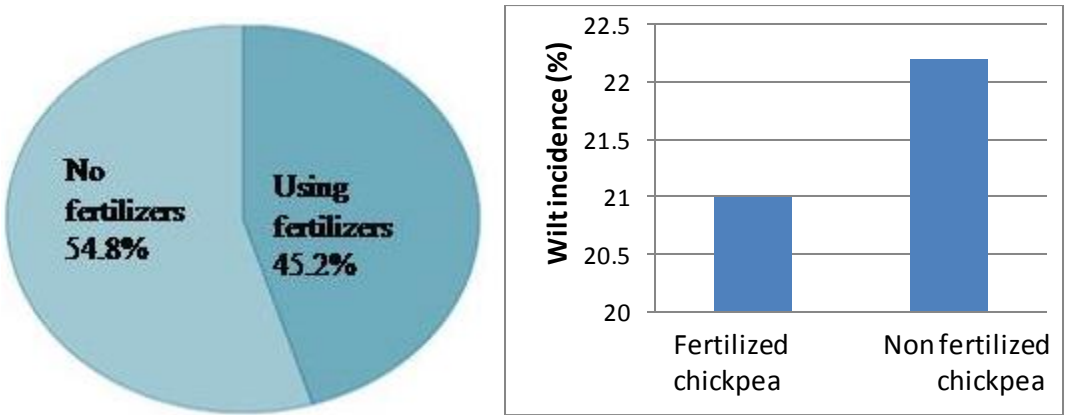


Fig. 15. Relationship between chickpea wiltincidence and seeding rate

4.1.7. Relationship betweenchickpeawilt incidence and fertilizers

The questionnaire results indicated that 54.8% of the farmers did not apply fertilizers while 45.2% of the farmers applied urea at the rate of 43kg N/ha (Fig. 16A). In addition, very few farmers used a single spray of foliar fertilizer at the rate of 4.7 L/ha. Use of fertilizers reduced incidence of wilt disease by only 1.2%(Fig. 16B).



A B

Fig.16. Percentage of farmers using fertilizers in chickpea fields (A) and wilt incidence in fertilized and non-fertilized fields (B).

4.1.8. Relationship between chickpea wilt incidence and wateringinterval

In the northern states farmers used to grow chickpea on residual soil moisture after the flood water of the Nile River subsides. Recently, however, theyshifted to pumps to irrigate their crops from the River Nile. In the Gezira the crop is grown as an irrigated crop and the watering intervals range from 7 to 21 days, with the 15-day interval as themost often used by farmers.

Disease incidence increased with increase in watering intervals.The disease incidence increased by 8 and 6% when the crop was watered every 2 and 3 weeks, respectively as compared to 1-week interval. (Table 10 and Fig. 17).

Table 10. Chickpea wilt incidence in relation to watering interval

watering intervals	Wilt incidence (%)
One week	16
Two weeks	24
Three weeks	22

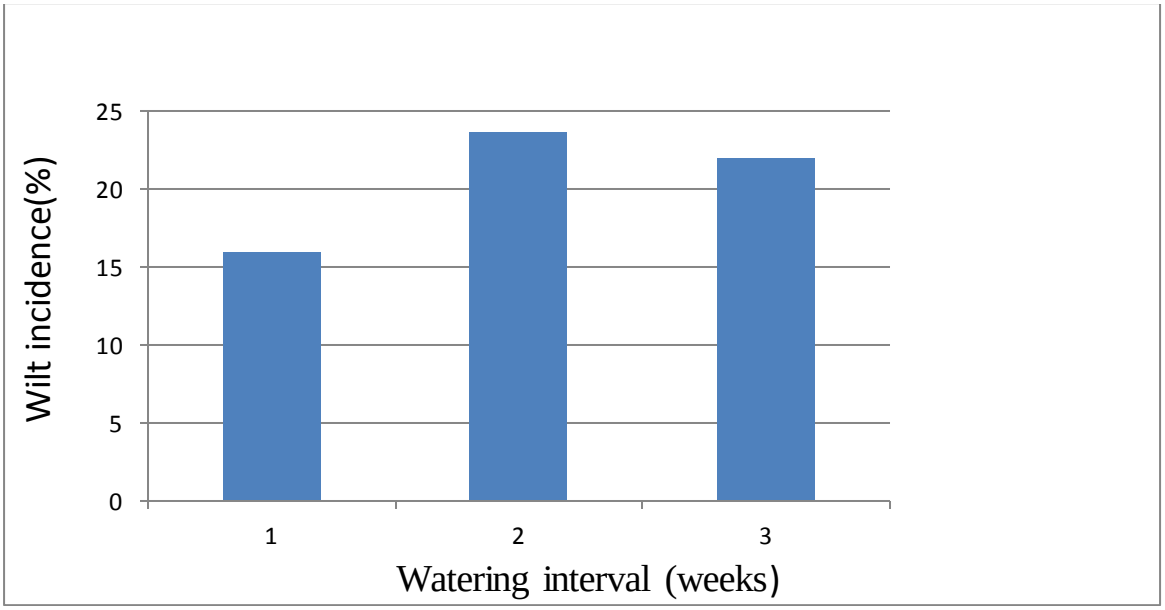


Fig. 17. Relationship between chickpea wilt incidence and watering interval

4.1.9. Effects of climatic conditions on chickpea Fusarium wilt incidence season 2011/2012

The climatic data (temperature and rainfall) for the states of Sudan from which samples were collected were recorded in Table 11. In winter season in Sudan, rains were rare and negligible. The temperatures recorded for the surveyed States during winter season 2011/12 from October until January, indicates that in October the temperatures were higher than in late December. When correlating the sowing dates with wilt incidence, the results indicated that disease incidence was higher in early sowings than in late sowings (Fig. 14). This indicates that there is a positive correlation between high temperatures and chickpea wilt incidence. The disease is lower in late sowings as a result of the decrease in temperatures.

The Relative humidity had no direct effects on disease incidence, as the rainfall is usually known to be rare and negligible during winter season in Sudan.

Table 11. Climatic data for Sudan statesfrom which wilted samples were collected (season 2011/2012)

Parameter	**Month**	Northern	River Nile	Khartoum	Gezira
AverageTemprature (C˚)	October	29.4	31.7	32.4	30
	November	23.5	26.8	28.1	27.3
	December	19.3	23.1	24.5	24.3
	January	17.6	21.4	23.2	23.6
	February	19.6	23.1	25	25.3
Average Relative Humidity(%)	October	25	25	28	47
	November	32	30	27	35
	December	36	35	30	35
	January	35	33	27	32
	February	30	25	22	25
Average rainfall (mm)	October	0.1	4.3	4.8	16
	November	0.5	Nil	0.7	1.5
	December	Nil	Nil	Nil	Nil
	January	Nil	Nil	Nil	Nil

4.2. Variability among *Fusarium oxysporum* f. sp. *ciceris* isolates

4.2.1. Cultural and morphological variability

In general, the fungus growth of all Sudanese isolates on PDA cultures initially appears as white feathery or cottony growth as shown in Fig. 18. The fungus growth of the Syrian and Lebanese isolates were feathery and cottony, respectively.

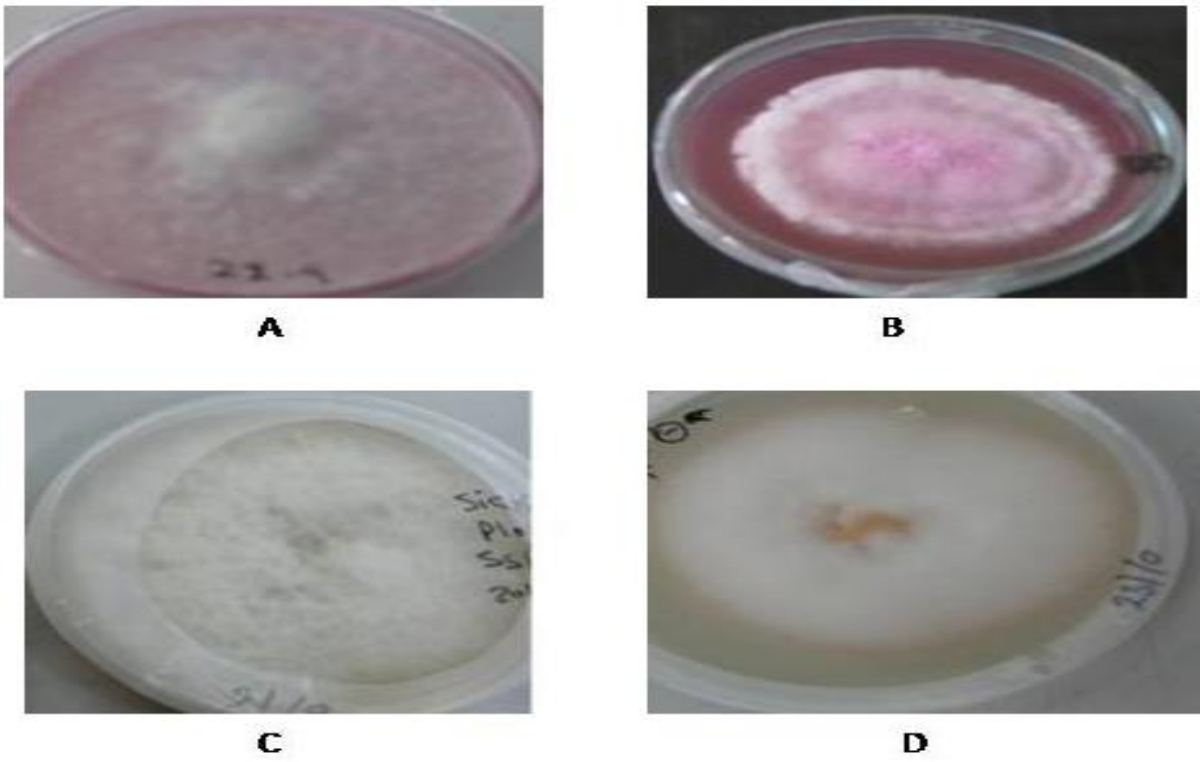


Fig. 18. Growth of *Fusarium oxysporum* f sp *ciceris*isolates on PDA. A= feathery growth of Sudanese isolates, B= cottony growth of Sudanese isolates, C= feathery growth of Syrian isolates, D= cottony growth of Lebanese isolates.

There are distinct differences between isolates in colony growth, diameter andcolor andsize and shapesof conidia and chlamydospores abundance

4.2.1.1. Differences in colony color and diameter

The isolates cultures were incubated at 25±2ºC for 7 days.The isolates were clustered into three groups according to their growth. Initially the growth of all Sudanese isolates on PDA started as white and changed with time into white pinkish feathery growth or white pinkish cottony growthor white pinkish feathery growth that change to violet by time.The cultures of Syrian and Lebanese isolates gave white feathery and cottony growth with violet and orange discoloration at the adaxial surface of the plate, respectively (Fig.19). The first group(A) with fast growing mycelium 70-90 mmand mean colony diameter of 85mmaccommodated 54isolates that were obtainedfromCentral Gezira, Kassala, River Nile, Khartoum and Sennar States. This group of isolates represented about 71% of the total Sudanese tested isolates. Thirteen isolateswith slow growing mycelium 40-49 mmand mean colony diameter of 44mm belonged to group B and they were collected from Northern state, north and central Gezira and River Nile State. This group of isolates represented about 17% of the tested isolates. The third group (C)with colony growth rate of 36-60mm and mean colony diameter of 48mm accommodated only 9 isolates from Gezira State and represented 12% of the tested isolates.The Syrian isolates (Fig. 19 D) were fast growing on PDA and their colony diameterreached 90 cm in 7 days incubation at 25˚C. However, under similar conditions,Lebanese isolates were slower and their myceliumgrowth diameter was 74-80mm(Fig. 19 E and Table 12). The Syrian and Lebanese isolates represented about 15% of all the tested isolates.

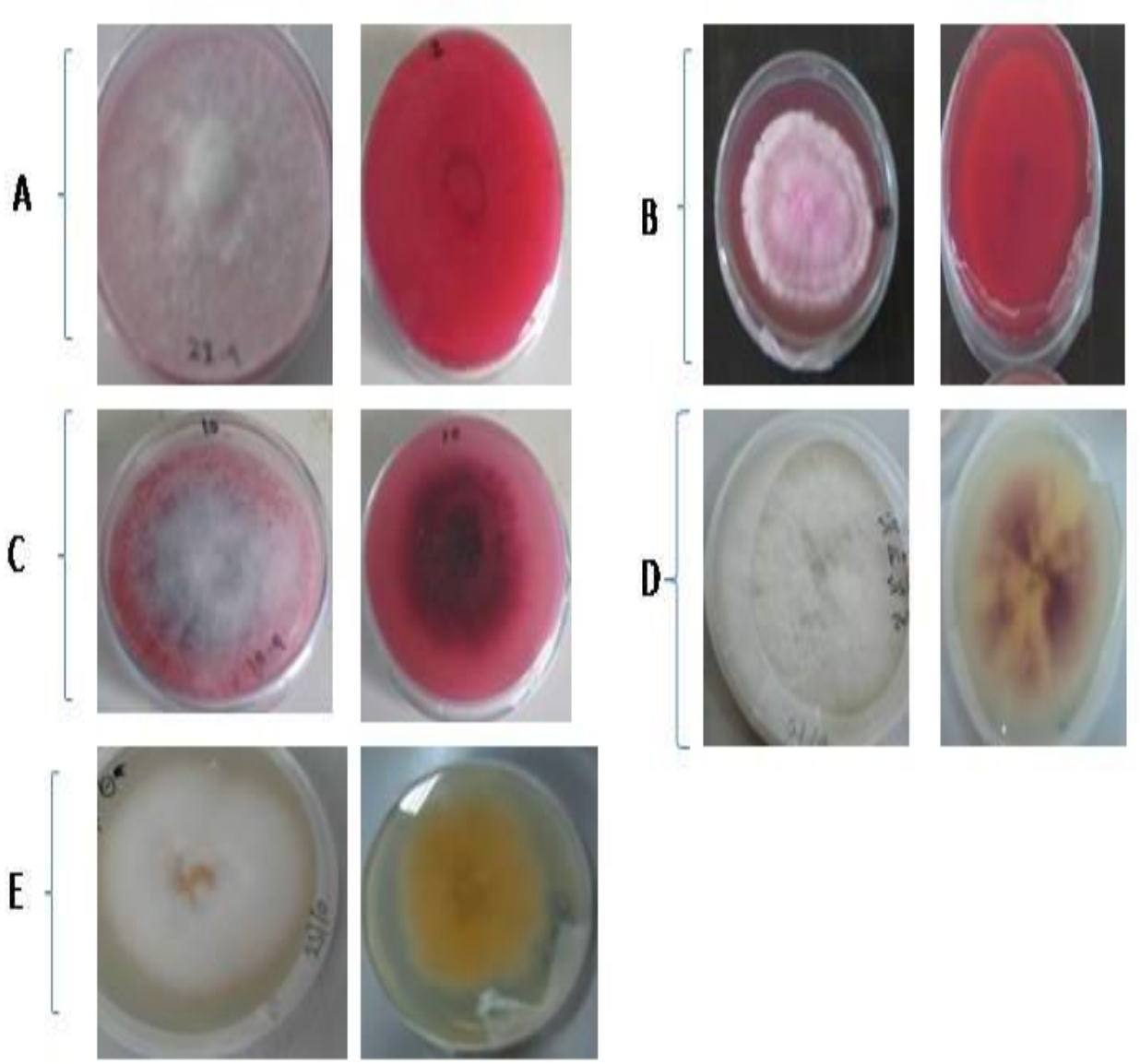


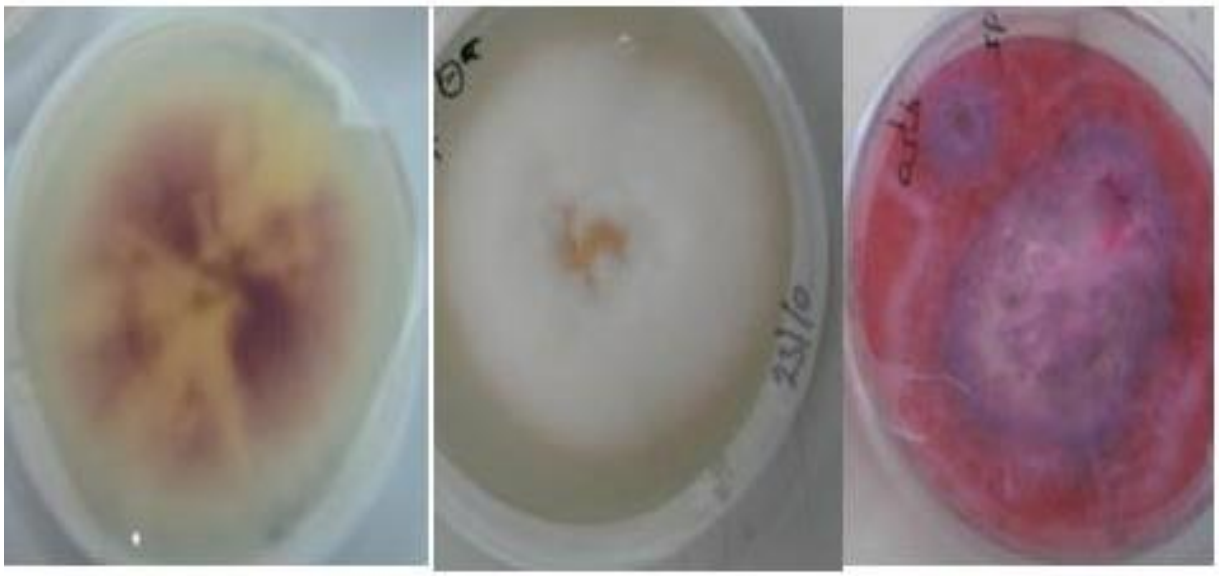
Fig. 19. Colony growth of *Fusarium oxysporum* f sp *ciceris* on PDA for 7 days.**A**=Fast growing isolates from Gezira,Sennar,Khartoum,River Nile and Kassala States.**B**=Very slow growing isolates from Northern, north Gezira and River Nile States.**C**=Slow growing isolates from Central Gezira. **D**=Syrian isolates. **E**=Lebanese isolates

Table12. Diameterof mycelial growth of representative *Foc*isolates (mm)

Isolates group	Isolates	Range of mycelium diameter (mm)	Isolates mean diameter (mm)	Group mean diamerter (mm)
A	New Halfa	90-90	90.0	84.88
	Daressalam	66-72	69.5	
	Hudeiba	90-90	90.0	
	Sennar	90-90	90.0	
B	Alburgeig	48-50	49.0	44.13
	Aldibeba	42-48	46.0	
	Barbar	40-44	41.0	
	Alburgeig	40-42	40.5	
C	Glasshouse	58-60	58.5	47.25
	Bagadi	50-56	52.0	
	Masalmiya	40-46	42.5	
	Altalha	30-38	36.0	
D	Syria 1	90-90	90.0	90.00
	Syria 2	90-90	90.0	
	Syria 3	90-90	90.0	
	Syria 4	90-90	90.0	
E	Lebanon1	70-80	73.0	75.13
	Lebanon2	76-82	80.0	
	Lebanon3	70-80	74.5	
	Lebanon4	70-80	73.0	
CV%			3.93	10.25
LSD			4.02	10.5

The diameter of the mycelia growth of the Sudanese isolates group A wassignificantlylarger than that of group B and C isolates, but they were not significantly different from the Syrian and Lebanese isolates (Table 12).There was no significant difference in the diameter of mycelia growth between group B and C isolates. However, they were significantly different from the Syrian and Lebanese isolates.

The cultures of the Syrian isolates had violet pigmentationon the lower surface, while the Lebanese isolates developed orange pigmentation on the media. Only the isolates from Altalha area in Sudan developed dark violet pigmentation that was very distinct from the violet pigmentation of the Syrian isolates (Fig. 20).



A B C

Fig. 20. Colony growth and pigmentation of (A) Syrian, (B) Lebanese and (C)Sudanese (Altalha)*Fusarium oxysporum* f sp *ciceris* isolates

4.2.1.2Differences in conidia sizes

Fungal hyphae of all isolates were septate and profusely branched. Microconidia are borne singly on simpleshort conidiophores, arising laterally on the hyphae. Microconidia and macroconidia are generally sparseon solid media and have variable size and septation within and between isolates (Fig 21). Microconidia are oval to cylindrical, straight to curved 0-2 septate and measure 3.75-12.9 x 2.1- 3.4 μm (Fig 21,Fig. 23 and Table 13) . Macroconidia are thin walled, 3-5 septate, fusoid,pointed at one or both ends and measure 17.5- 42.5 x 2.5-6.25μm(Fig. 21, 23, 24, 25, 26, 27and Table 14). Chlamydospores formed only in 15-day-old cultures of the isolates that belong to group A and they were smooth or rough walled, may form singly, in pairs, or in chains(Fig. 22 and Fig.23).

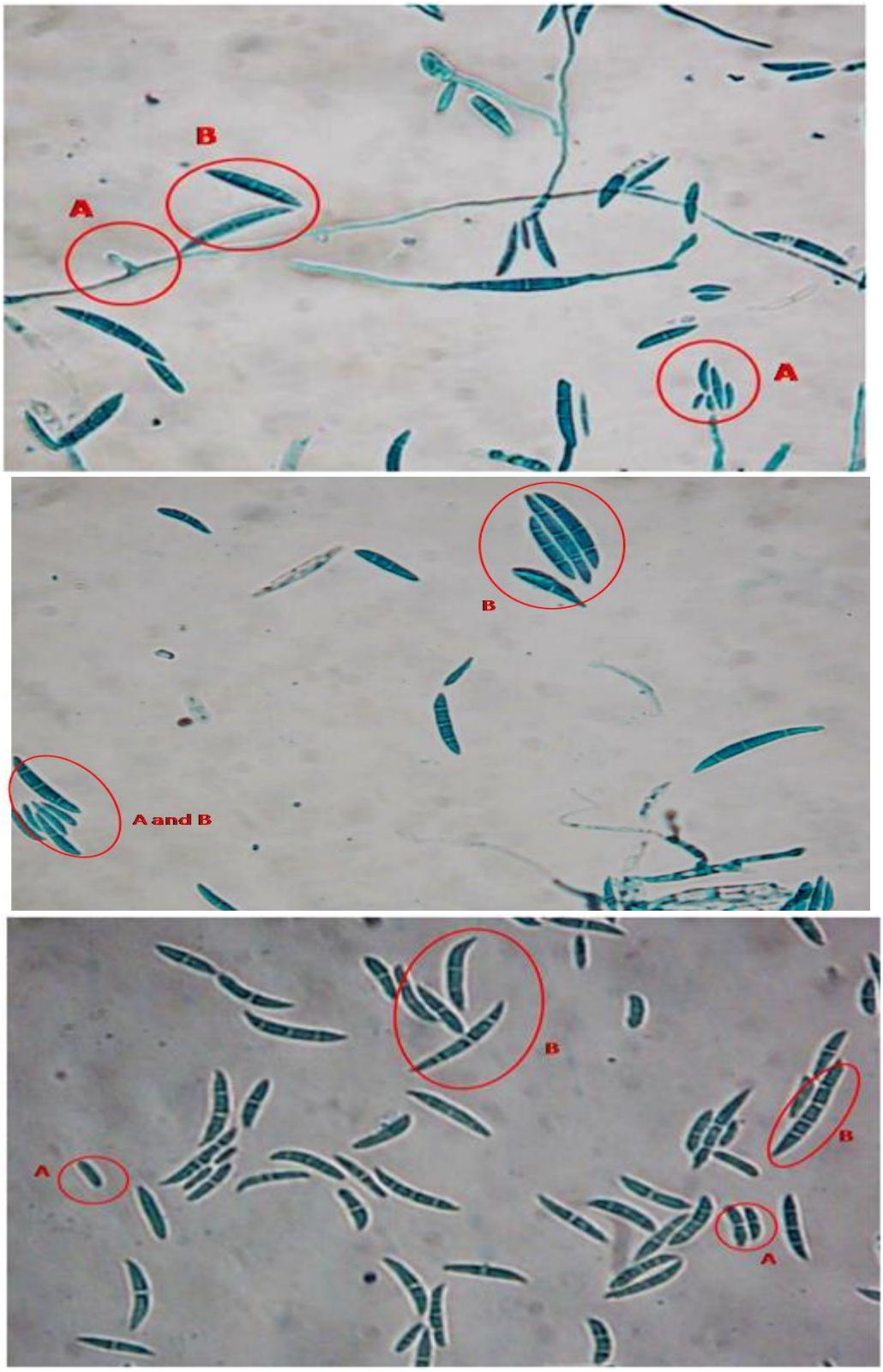


Fig. 21. Microconidia (A) and Macroconidia (B) of *Fusarium oxysporum* f. sp.*Ciceris*isolates

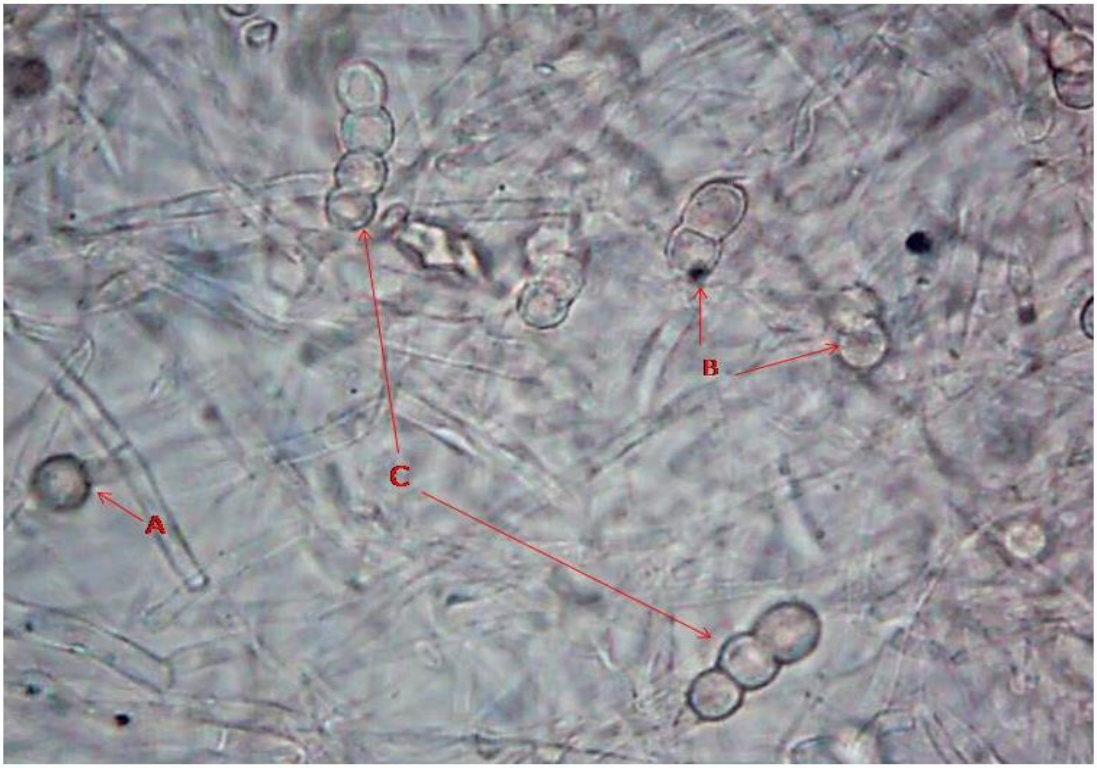


Fig.22.*Fusarium oxysporum* f. sp.*ciceris* Chlamydospores (A) singly, (B) in pairs and (C) in chains

The length of the microconidia of group Aisolates from Gezira, Sennar, New Halfa and Hudeiba sick plotranged from 6.25 -12.8µm. However, the width ranged from 2.1-5.0 µm (Table 13). The macroconidia length ranged from19.29-30.0µm in length, while thewidth ranged from2.5-6.25µm (Table 14). Chlamydospores were abundantonly in these isolates(Fig.23)

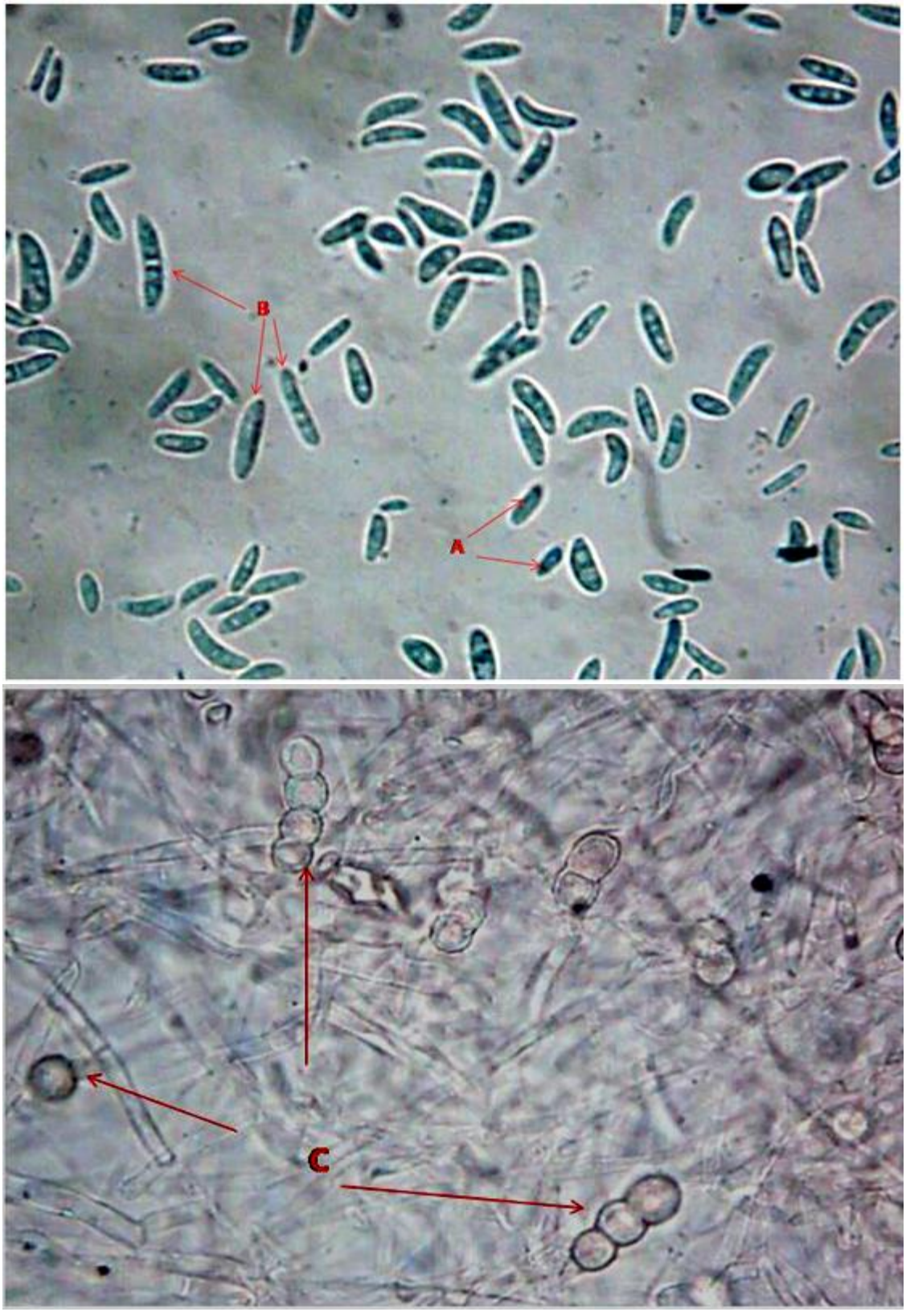


Fig. 23. Microconidia (A), macroconidia(B) and chlamydospores (C) of Sudanese isolates group A

Group B accommodated isolates from Aldibaiba, Alburgaig and Barbar. In this group, the microconidia were 5-12.8µm long and 2.1-5.0µm wide (Table 13). The macroconodia were 17.5-35.0µm long and 2.5-5.0 µm wide (Table 14). Macroconidiaof group B were longerand slender than those of group A isolates (Fig. 24 and Table 14).

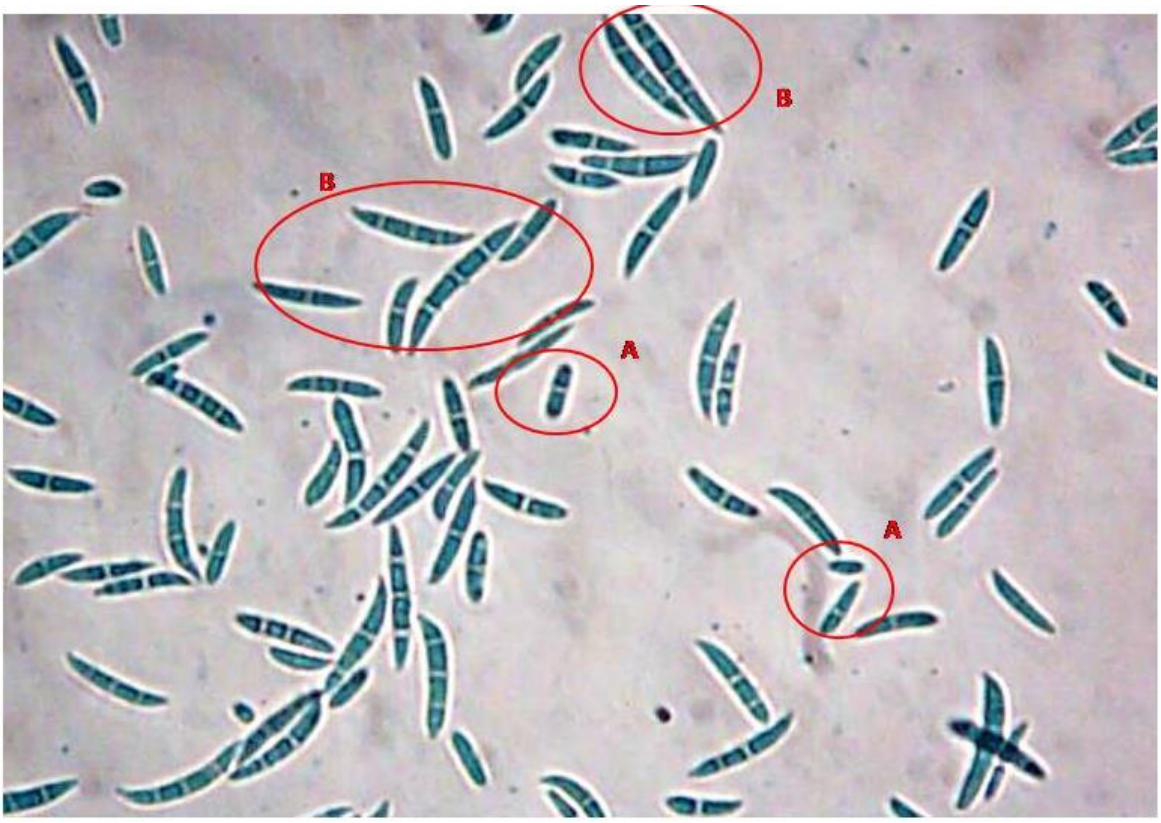


Fig. 24. Microconidia (A) and macroconidia (B) of the Sudanese isolates group B

The microconidia of group C isolates represented by Altalha, Bagadi, Masalamiyaand Wad medani (GH)were 3.75-12.9µm long and 2.14-3.75µm wide (Table 13). The macroconidia of this group were 20-42.5µm longand 2.5-5.0 µm wide(Table14). The macroconidia in this group were longer than those in group A and B (Fig. 25).

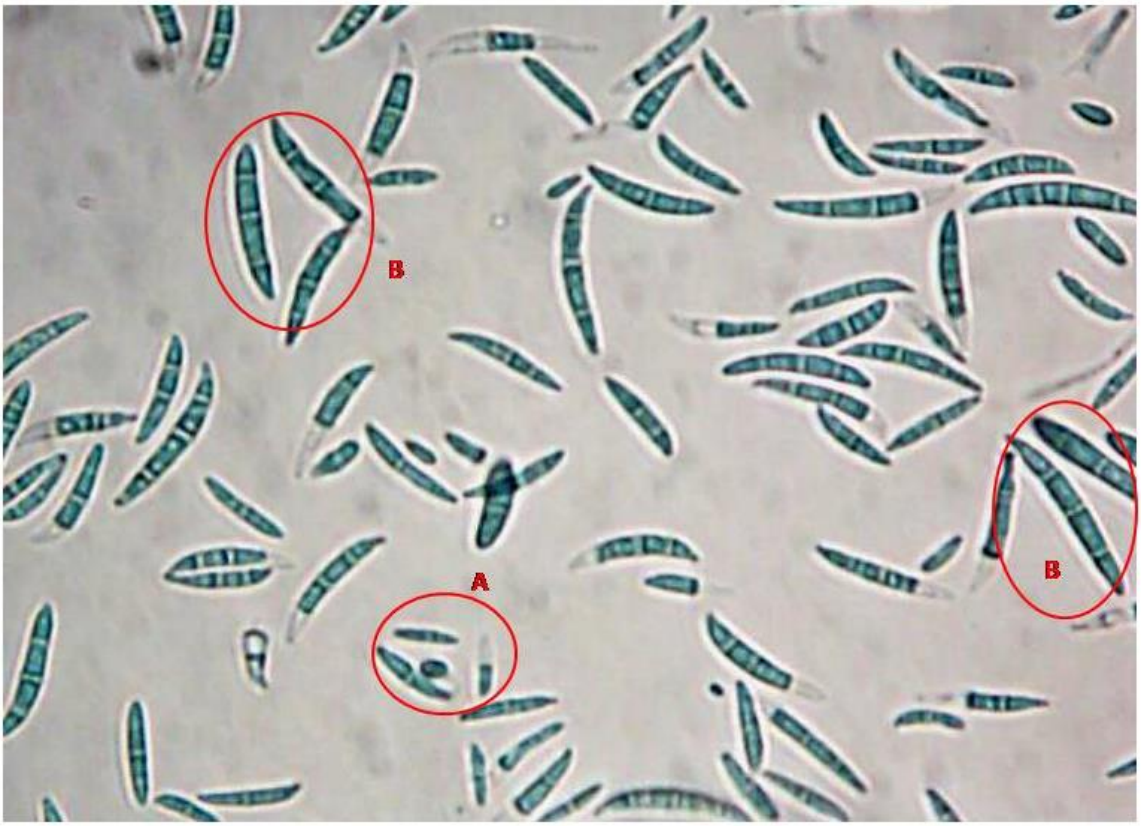


Fig. 25. Microconidia (A) and macroconidia (B) of Sudaneseisolates group C

Table 13. Size of microconidia (µm) of some Sudanese, Syrian and Lebanese isolates

Group	Isolate	Microconidia length(µm)			Group mean	Isolate	Microconidia width(µm)			Group mean
		Min	max	Mean			Min	Max	Mean	
A	New Halfa	7.50	12.5	10.25	10.04	NewHalfa	2.5	3.7	2.62	2.81
	Dar Esalam	6.25	12.5	08.95		DarEsalm	2.5	5.0	2.88	
	Hudeiba	8.57	12.8	10.90		Hudeiba	2.1	3.4	2.72	
	Sennar	8.60	12.8	10.07		Sennar	2.1	3.4	3.01	
B	Alburgeig1	6.43	12.8	10.48	09.32	Alburgeig1	2.1	3.4	2.75	2.65
	Aldibeba	6.43	12.8	09.80		Aldibeba	2.2	2.2	2.20	
	Barbar	5.00	12.5	07.63		Barbar	2.5	3.75	2.63	
	Alburgeig2	5.00	12.5	09.38		Alburgeig2	2.5	5.0	3.00	
C	Glasshouse	6.43	12.9	10.42	09.03	Glashouse	2.14	3.43	2.66	2.79
	Bagadi	3.75	10.0	07.88		Bagadi	2.50	2.50	2.50	
	Masalmiya	4.50	10.0	07.53		Masalmya	3.40	3.40	3.40	
	Altalha	6.43	12.8	10.27		Altalha	2.50	3.75	2.63	
D	Syria 1	7.50	10.0	09.25	09.83	Syria 1	2.5	3.75	2.88	2.59
	Syria 2	8.57	12.8	10.40		Syria 2	2.5	3.75	2.88	
	Syria 3	7.50	10.0	09.25		Syria 3	2.3	2.3	2.3	
	Syria 4	8.57	12.8	10.40		Syria 4	2.3	2.3	2.3	
E	Lebanon 1	8.57	12.0	10.37	10.39	Lebanon 1	3.4	3.4	3.4	3.42
	Lebanon 2	8.60	12.0	10.41		Lebanon 2	3.43	3.43	3.43	
	Lebanon 3	8.60	12.0	10.41		Lebanon 3	3.43	3.43	3.43	
	Lebanon 4	8.57	12.0	10.37		Lebanon 4	3.4	3.4	3.4	
CV%				20.5	9.93				17.7	11.19
LSD				1.74	1.44				0.44	0.48

Table 14. Size of macroconidia (µm) of some Sudanese, Syrian and Lebanese isolates

Group	Isolates	Macroconidia length (µm)			Mean	Isolate	Macroconidia width(µm)			Mean
		Min	Max	Mean			Min	Max	Mean	
A	NewHalfa	23.75	30.00	27.12	25.32	NewHalfa	2.50	4.50	3.55	4.33
	DarEsalam	23.75	30.00	27.62		DarEsalam	2.50	6.25	4.50	
	Hudeiba	19.29	23.57	21.22		Hudeiba	4.29	5.50	4.96	
	Sennar	21.43	30.00	25.30		Sennar	4.30	4.30	4.30	
B	Alburgeig1	23.57	34.29	28.29	27.92	Alburgeig1	3.85	5.00	4.32	4.02
	Aldibeba	25.71	34.29	29.14		Aldibeba	3.40	3.40	3.40	
	Barbar	22.50	35.00	29.25		Barbar	3.75	5.00	4.50	
	Alburgeig2	17.50	35.00	25.00		Alburgeig2	2.50	5.00	3.88	
C	Glasshouse	21.43	34.29	28.71	29.57	Glasshouse	4.28	5.00	4.50	4.28
	Bagadi	20.00	42.50	28.25		Bagadi	2.50	5.00	4.25	
	Masalamya	23.57	38.57	29.57		Masalamya	3.75	5.00	4.00	
	Altalha	25.00	42.50	31.75		Altalha	4.28	5.00	4.36	
D	Syria 1	22.50	37.50	29.75	30.58	Syria 1	3.75	5.00	4.38	4.19
	Syria 2	22.30	38.57	31.41		Syria 2	3.40	4.30	4.02	
	Syria 3	22.50	37.50	29.75		Syria 1	3.75	5.00	4.38	
	Syria 4	22.30	38.57	31.41		Syria 2	3.40	4.30	4.02	
E	Lebanon1	21.40	34.29	25.62	25.63	Lebanon1	4.30	5.00	4.58	4.58
	Lebanon2	21.40	34.29	25.62		Lebanon2	4.20	5.10	4.59	
	Lebanon3	21.40	34.30	25.63		Lebanon1	4.30	5.00	4.58	
	Lebanon4	21.40	34.30	25.63		Lebanon2	4.20	5.10	4.59	
CV%				16.5	6.79				13.7	8.84
LSD				4.03	2.80				0.52	0.57

The microconidia for the Syrian isolates (group D) measured 7.5-12.8µm long and 2.3 - 3.75µm wide whereas the macroconidia measured 22.3-38.57µm long and 3.4-5.0 µm wide (Tables13 and 14).

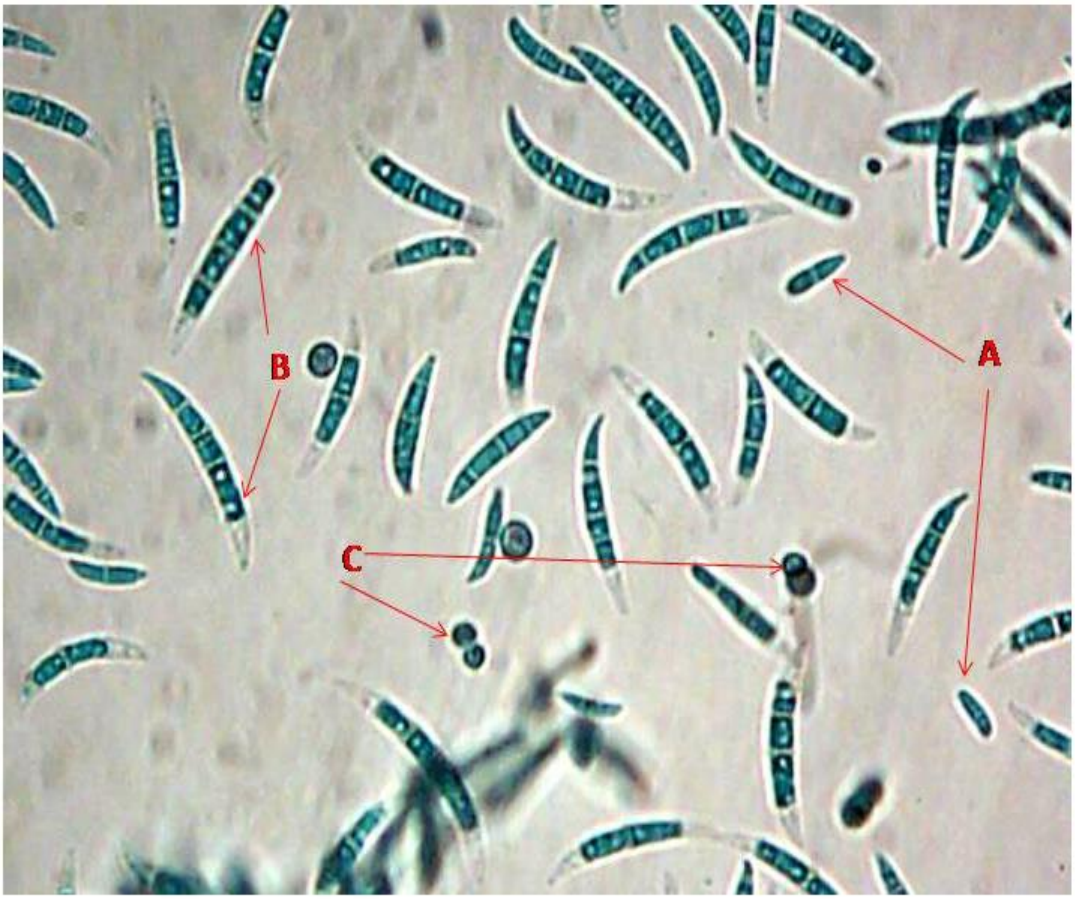


Fig. 26. Microconidia (A), macroconidia (B) and chlamudospres (C) of Syrian isolates

For the isolates from Terbol of Lebanon (group E), the microconidia measured 8.5- 12.0µm long and 3.4 - 3.43µm wide.The macroconidia measured 21.4 - 34.3µmlong and4.2 - 5.1µm wide (Tables 13 and 14)

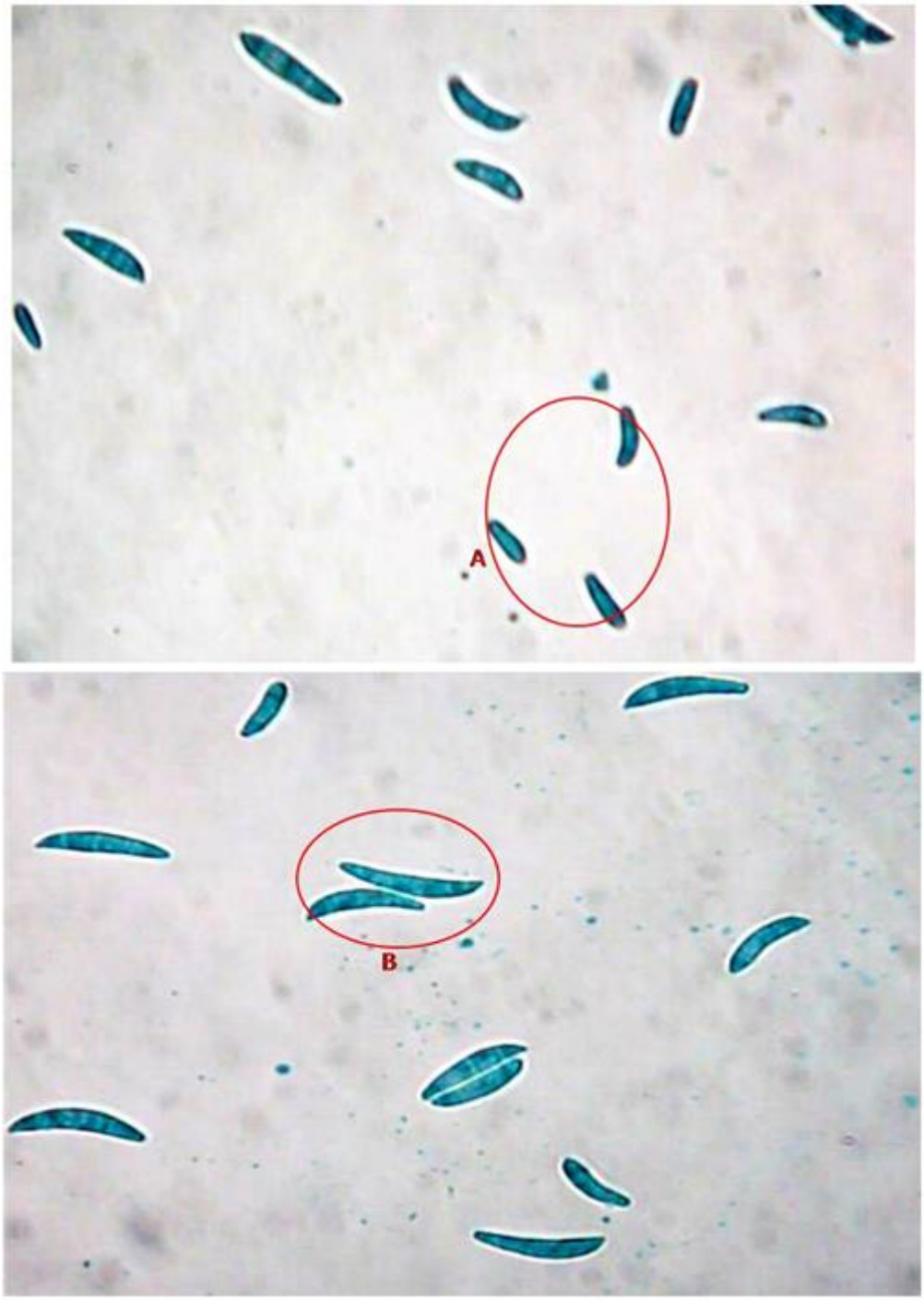


Fig. 27. Microconidia (A) and macroconidia (B) of Lebanese isolates

There were no significant differencies between micoconidia length and width of all the isolates. Microconidia length was in a range of 3.75-

12.9µm and the width of 2 .1- 5.0µm. The longest macroconidia were observed in Bagadi and Altalha isolates (group C), while the shortest were in Alburgaig and Hudeiba sick plot of group B and Aisolates, respectively. Daressalam isolates macroconidia were the widest smong all the isolates. The average length of macroconidia of group C isolates was longer than that of group A, B isolates and the shortest length was observed in group A isolates. Generally, the size of the macroconidia of the different isolates ranged from 17.5 -42.5µm in length and 2.5-6.25µm in width. Chlamydospores were observed only in central Gezira and Syrian isolates.

4.2.2. Pathogenic variability

The disease reaction of a set of differential chickpea cultivars (Table 15)to the 25 tested isolates was predominantly similar. All the cultivarsexcept Shendi were either resistant or moderatly resistant to the tested isolates. Shendi was susceptible to the disease when inoculated with all the isolates. Infected plants showed progressive foliar yellowing and drying of the older leaves, then wilting of the plantin 45 days after inoculation. The browning of vascular bundle of wilted plants was evident indicating diagnostic symptoms. Seven of the differential cultivars exhibited resistant reaction to all isolates while only Annigeri and Chafa were moderatly resistant. However only Shendi was sucseptible to all isolates and these results clearly indicated that the 25 isolates belong to the same race which issimilar to race 0 according to Halila and Strange (1997).

Table 15. Reaction of chickpea Differential varieties to 25 Sudanese *Foc* isolates.

Isolates	JG 62	BG 212	C 104	JG 74	CPS1	WR 315	Annigeri	Chafa	L550	Shendi
Um Jeer1	R	R	R	R	R	R	MR	MR	R	S
Um Jeer2	R	R	R	R	R	R	MR	MR	R	S
Um arfaya1	R	R	R	R	R	R	MR	MR	R	S
Um arfaya2	R	R	R	R	R	R	MR	MR	R	S
WadElabyadni1	R	R	R	R	R	R	MR	MR	R	S
WadElabyadani	R	R	R	R	R	R	MR	MR	R	S
Wad haggar1	R	R	R	R	R	R	MR	MR	R	S
Wad haggar2	R	R	R	R	R	R	MR	MR	R	S
El irayek	R	R	R	R	R	R	MR	MR	R	S
Hilat Farah1	R	R	R	R	R	R	MR	MR	R	S
Hilat Farah2	R	R	R	R	R	R	MR	MR	R	S
Hilat Farah3	R	R	R	R	R	R	MR	MR	R	S
Altalbab	R	R	R	R	R	R	MR	MR	R	S
Hudeiba SP1	R	R	R	R	R	R	MR	MR	R	S
HudeibaSP2	R	R	R	R	R	R	MR	MR	R	S
Hudeiba SP3	R	R	R	R	R	R	MR	MR	R	S
Sennar1	R	R	R	R	R	R	MR	MR	R	S
Sennar2	R	R	R	R	R	R	MR	MR	R	S
Shambat SP1	R	R	R	R	R	R	MR	MR	R	S
Shambat SP2	R	R	R	R	R	R	MR	MR	R	S
New Halfa1	R	R	R	R	R	R	MR	MR	R	S
New Halfa2	R	R	R	R	R	R	MR	MR	R	S
New Halfa3	R	R	R	R	R	R	MR	MR	R	S
New Halfa4	R	R	R	R	R	R	MR	MR	R	S
New Halfa5	R	R	R	R	R	R	MR	MR	R	S

R=11-20% wilted plants, MR=21-40% wilted plants, S=41-80% wilted plants. SP= sick plot

4.3. Genetic diversity and race analysis of *Fusarium oxysporum* f.sp.*ciceris* isolates

4.3.1. *Fusarium oxysporum* f. sp. *ciceris*-specific PCR protocol for pathogenic *Foc*

The PCR assays using primer sets FOCP1 and FOCP2 amplified a single band of genomic DNA of *F. oxysporum* f. sp. *ciceris* isolates representing isolates from Sudan, Syria and Lebanon.Amplification of FOCP1 and FOCP2 were at sizes 160 and 158 bp, respectively (Table 16 and Fig.28). This clearly indicates that all the tested isolates from different areas in Sudan are pathogenic and belongs to *Fusarium oxysporum* f. sp.*ciceris*.

Table 16. Amplicon size of FOCP1 and FOCP2 primers

Primer type	Primer name	Amplicon size (bp)
Specific for pathogenic *Foc*	FOCP1	160
	FOCP2	158

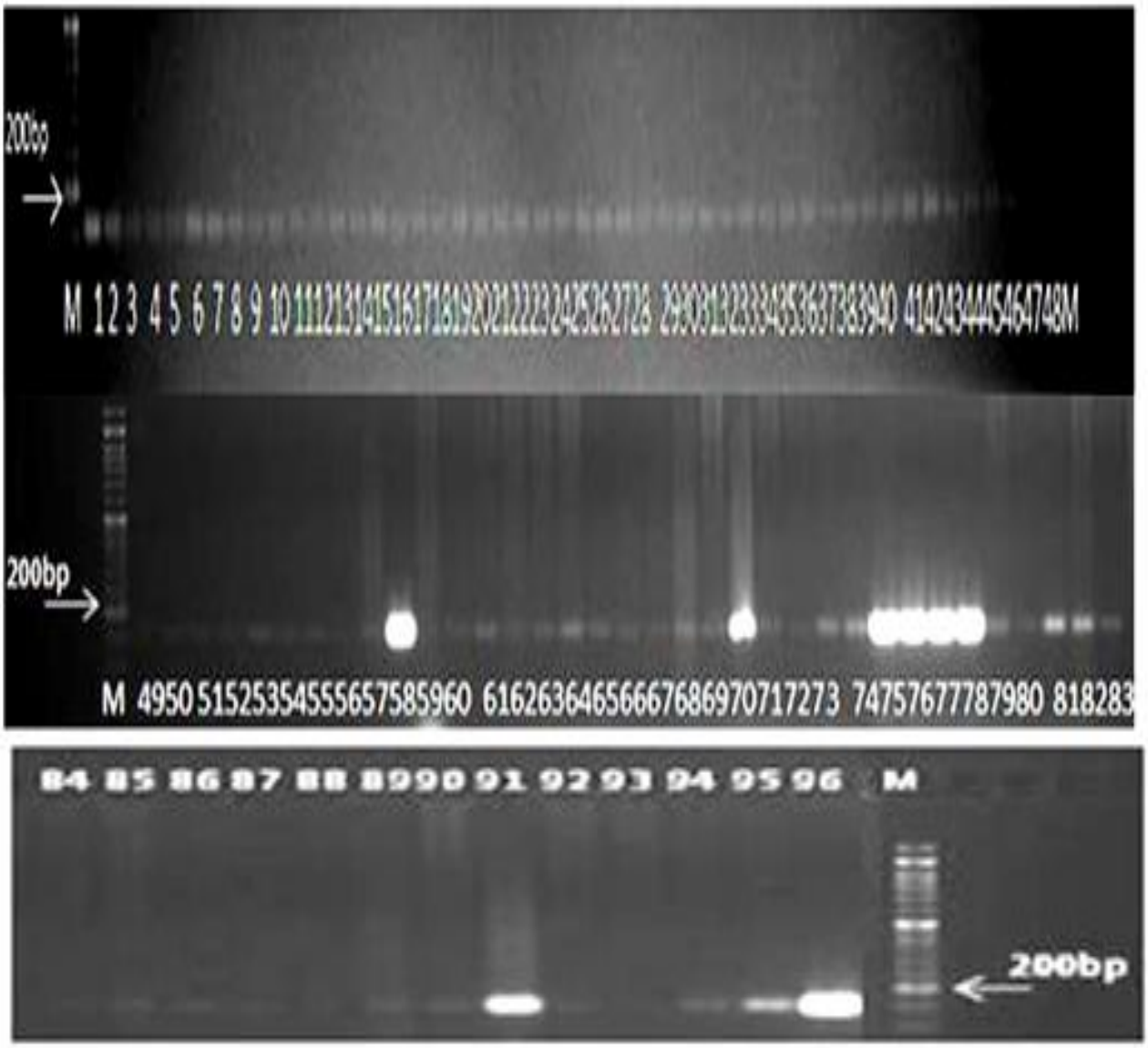


Fig. 28.DNA profile generated by **FOCP1**primer; M= marker-50bp; Lanes:1, 44 and 47 (Shambatisolates); 2,5-7, 13, 16, 18-20, 22-24, 28, 29, 36-38,41,42,45,46,48,51,57, 58(NewHalfa isolates); 3,4,8-12,14,15,21,26,27,30-35,39,40,49, 50, 52-54,59,61,63-70,72,77-79,81-82, 85(Gezira isolates);17,25 (Sennar isolates); 43,55,56,60 (Hudeiba isolates); 62, 71, 73, 86-89 (Terbol, Lebanon); 74-76,83, 84(Burgeig and Bushariya isolates);80 Barbar isolate; 90-96 (ICARDA sick plot, Syria)

4.3.2. Random amplified polymorphic DNA(RAPD) primers analysis

All amplification reactions of the RAPD primers (OPF12, OPF16, OPIO1 and OPFO6) generated 14-18 polymorphic DNA bands with all the tested isolates (Fig. 29, 30, 31 and 32 and Table 17).

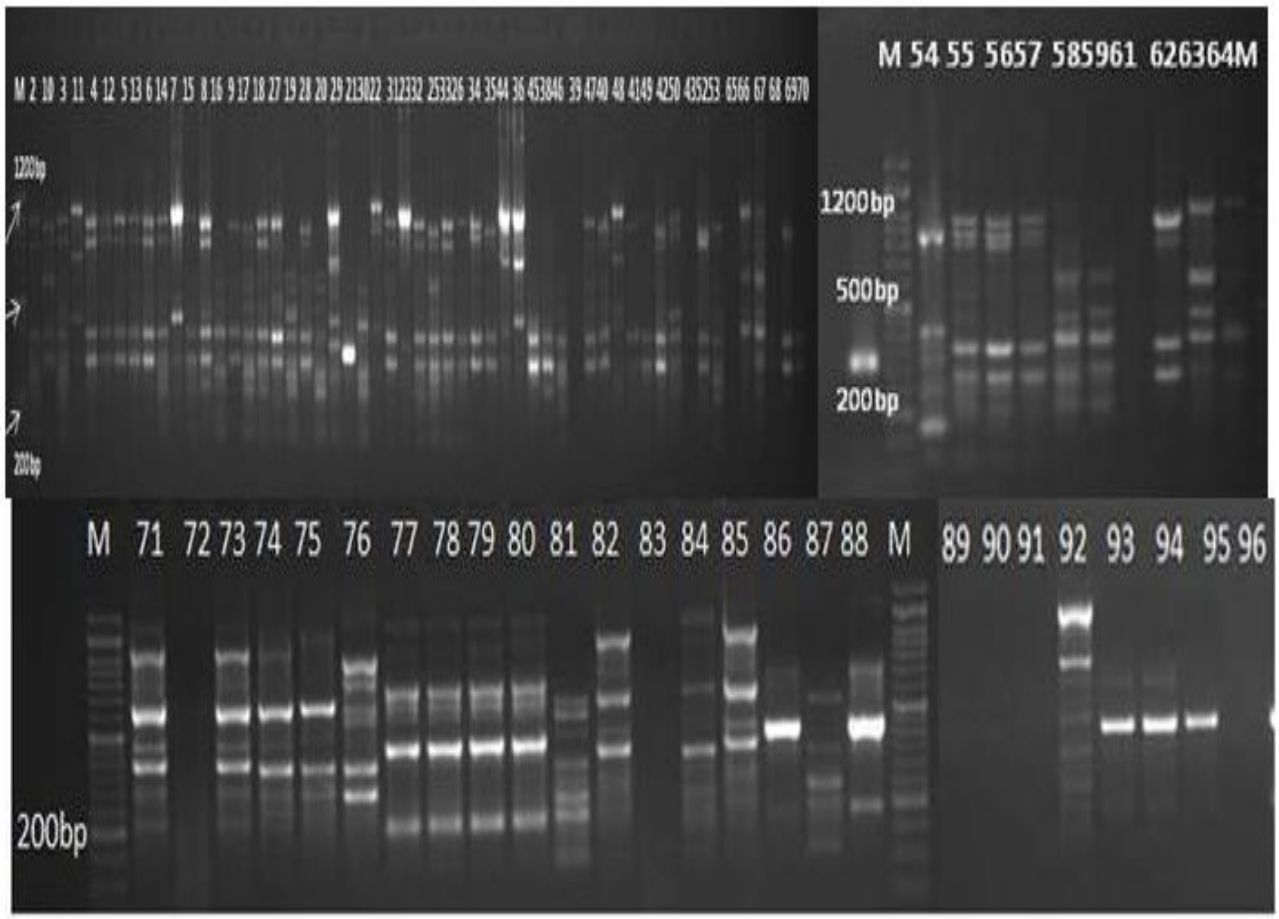


Fig. 29.DNA profile generated by RAPD (**OPF12**) primer ; M= marker-50bp; Lanes: 1, 44 and 47 (Shambat isolates); 2, 5-7, 13, 16, 18-20, 22-24, 28, 29, 36-38, 41, 42, 45, 46, 48, 51, 57, 58 (NewHalfa isolates); 3, 4, 8-12, 14, 15, 21, 26, 27, 30-35, 39, 40, 49, 50, 52-54, 59, 61, 63-70, 72, 77-79, 81-82, 85 (Gezira isolates); 17, 25 (Sennar isolates); 43, 55, 56, 60 (Hudeiba isolates); 62, 71, 73, 86-89 (Terbol, Lebanon); 74-76, 83, 84 (Burgeig and Bushariya isolates); 80 Barbar isolate; 90-96 (ICARDA sick plot, Syria)

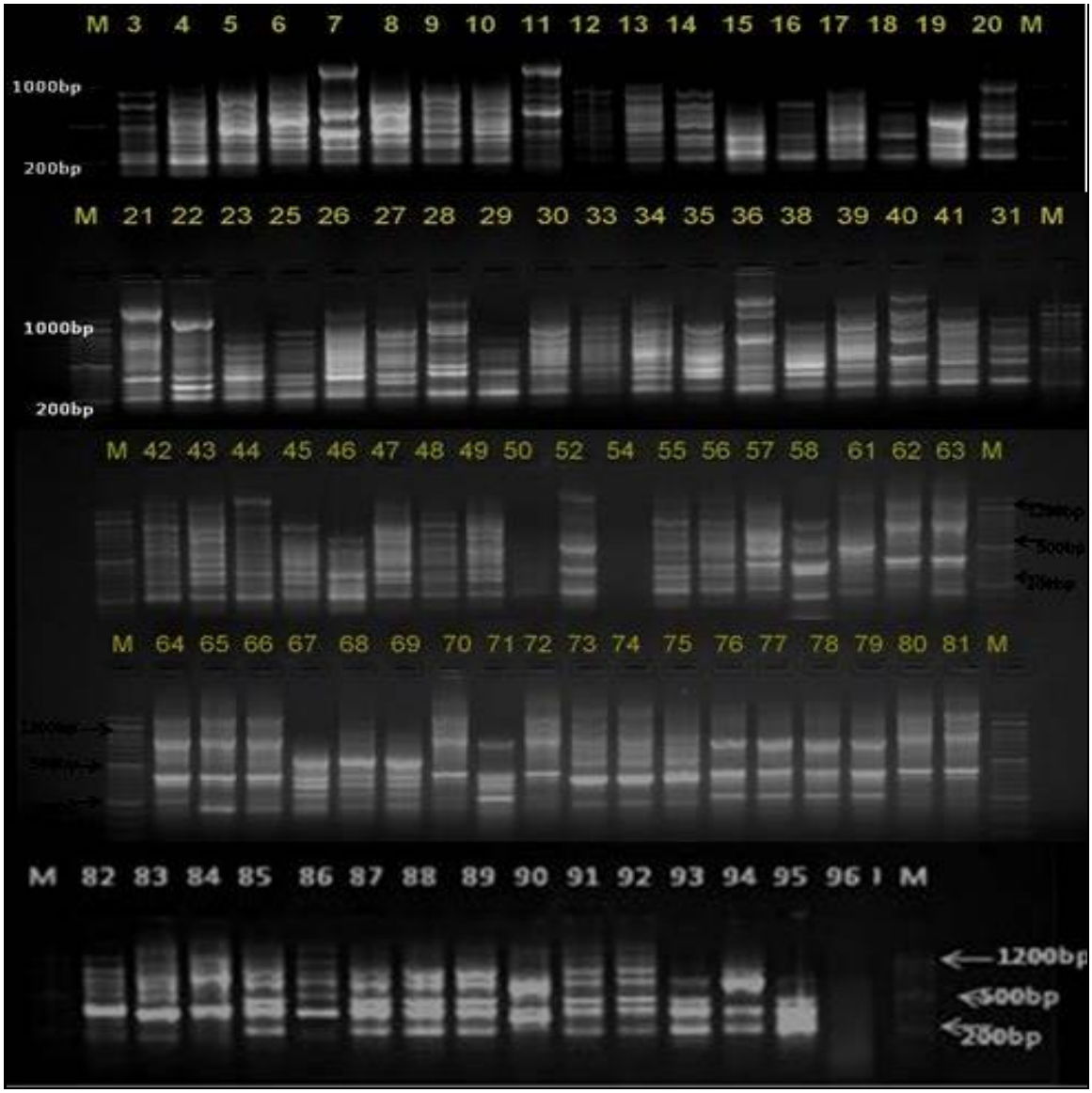


Fig. 30.DNA profile generated by RAPD (**OPIO1**) primer ; M= marker-50bp; Lanes: 1, 44 and 47 (Shambat isolates); 2, 5-7, 13, 16, 18-20, 22-24, 28, 29, 36-38, 41, 42, 45, 46, 48, 51, 57, 58 (NewHalfa isolates); 3, 4, 8-12, 14, 15, 21, 26, 27, 30-35, 39, 40, 49, 50, 52-54, 59, 61, 63-70, 72, 77-79, 81-82, 85 (Gezira isolates); 17, 25 (Sennar isolates); 43, 55, 56, 60 (Hudeiba isolates); 62, 71, 73, 86-89 (Terbol, Lebanon); 74-76, 83, 84 (Burgeig and Bushariya isolates); 80 Barbar isolate; 90-96 (ICARDA sick plot, Syria**)**

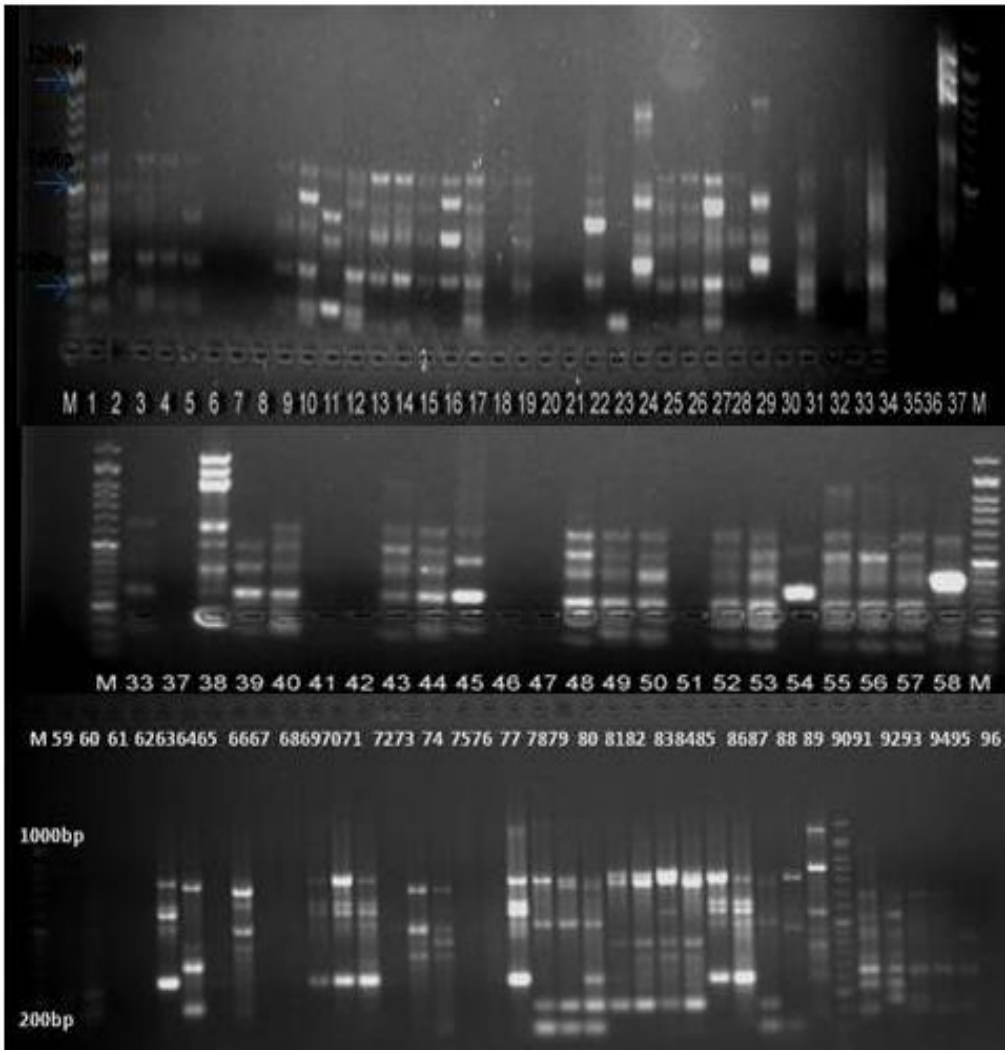


Fig. 31.DNA profile generated by RAPD (**OPF16)**; M= marker-50bp; Lanes: 1, 44 and 47 (Shambat isolates); 2, 5-7, 13, 16, 18-20, 22-24, 28, 29, 36-38, 41, 42, 45, 46, 48, 51, 57, 58 (NewHalfa isolates); 3, 4, 8-12, 14, 15, 21, 26, 27, 30-35, 39, 40, 49, 50, 52-54, 59, 61, 63-70, 72, 77-79, 81-82, 85 (Gezira isolates); 17, 25 (Sennar isolates); 43, 55, 56, 60 (Hudeiba isolates); 62, 71, 73, 86-89 (Terbol, Lebanon); 74-76, 83, 84 (Burgeig and Bushariya isolates); 80 Barbar isolate; 90-96 (ICARDA sick plot, Syria)

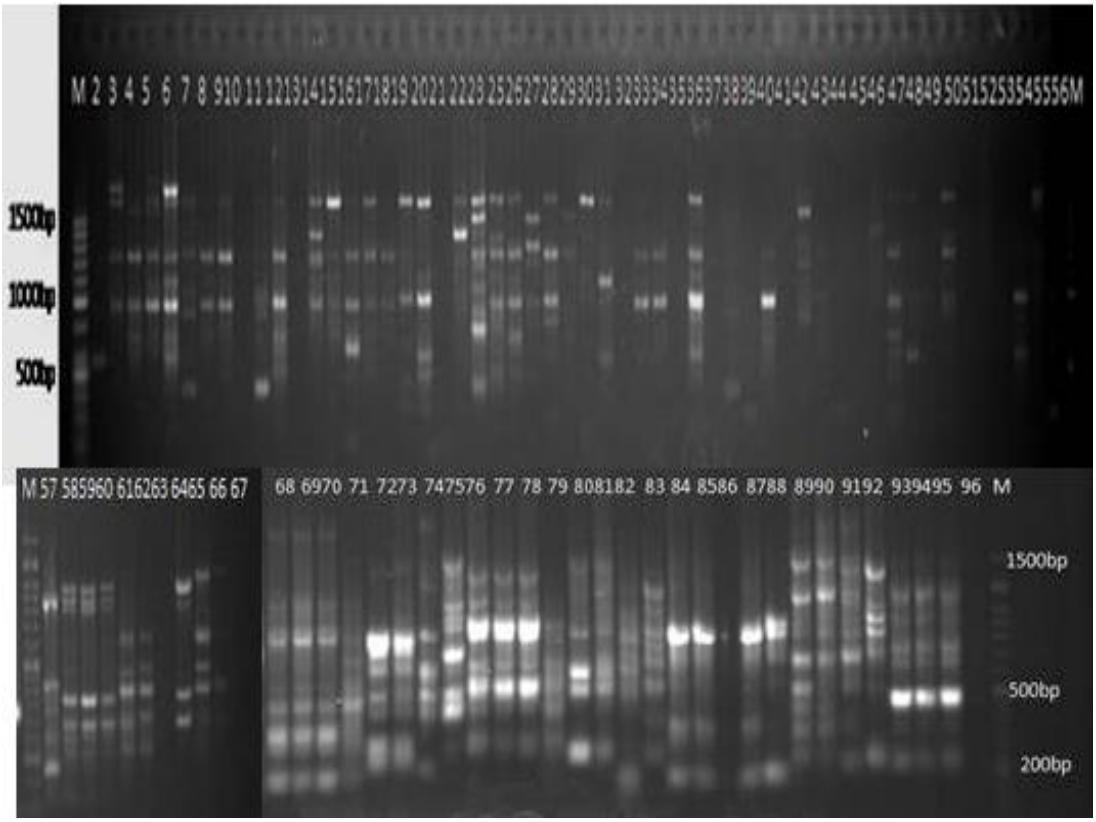


Fig. 32.DNA profile generated by RAPD (**OPF06**) primer ; M= marker-50bp; Lanes: 1, 44 and 47 (Shambat isolates); 2, 5-7, 13, 16, 18-20, 22-24, 28, 29, 36-38, 41, 42, 45, 46, 48, 51, 57, 58 (NewHalfa isolates); 3, 4, 8-12, 14, 15, 21, 26, 27, 30-35, 39, 40, 49, 50, 52-54, 59, 61, 63-70, 72, 77-79, 81-82, 85 (Gezira isolates); 17, 25 (Sennar isolates); 43, 55, 56, 60 (Hudeiba isolates); 62, 71, 73, 86-89 (Terbol, Lebanon); 74-76, 83, 84 (Burgeig and Bushariya isolates); 80 Barbar isolate; 90-96 (ICARDA sick plot, Syria)

Table 17. Total number of bands generated by RAPD primers

Primer type	Primer name	Total number of bands	Amplicon size (bp)
RAPD	OPI-01	14	200-1500
	OPF-06	16	100-1700
	OPF-12	18	200-1500
	OPF-16	16	150-1700

4.3.3. Simple sequence repeats primers (SSRs)

The three SSR primers tested, MB18,MB17 and MB14, amplified reproducible bands with all the isolates. MB18 primer amplified products between 250 and 900 bpwhile MB17 amplified products between 250 and 300bp and for MB14 the amplification was between 400 and 600 bp (Fig33, 34, 35 and Table 18).

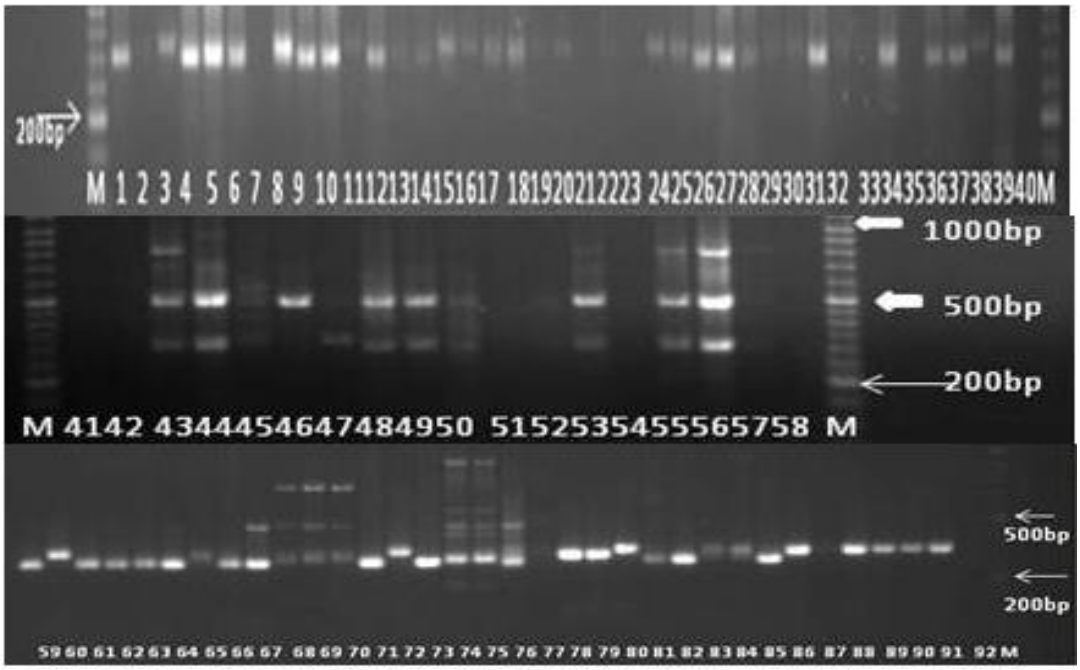


Fig. 33.DNA profile generated by SSR (**MB18)**primer; M= marker-50bp; Lanes: 1, 44 and 47 (Shambat isolates); 2, 5-7, 13, 16, 18-20, 22-24, 28, 29, 36-38, 41, 42, 45, 46, 48, 51, 57, 58 (NewHalfa isolates); 3, 4, 8-12, 14, 15, 21, 26, 27, 30-35, 39, 40, 49, 50, 52-54, 59, 61, 63-70, 72, 77-79, 81-82, 85 (Gezira isolates); 17, 25 (Sennar isolates); 43, 55, 56, 60 (Hudeiba isolates); 62, 71, 73, 86-89 (Terbol, Lebanon); 74-76, 83, 84 (Burgeig and Bushariya isolates); 80 Barbar isolate; 90-96 (ICARDA sick plot, Syria)

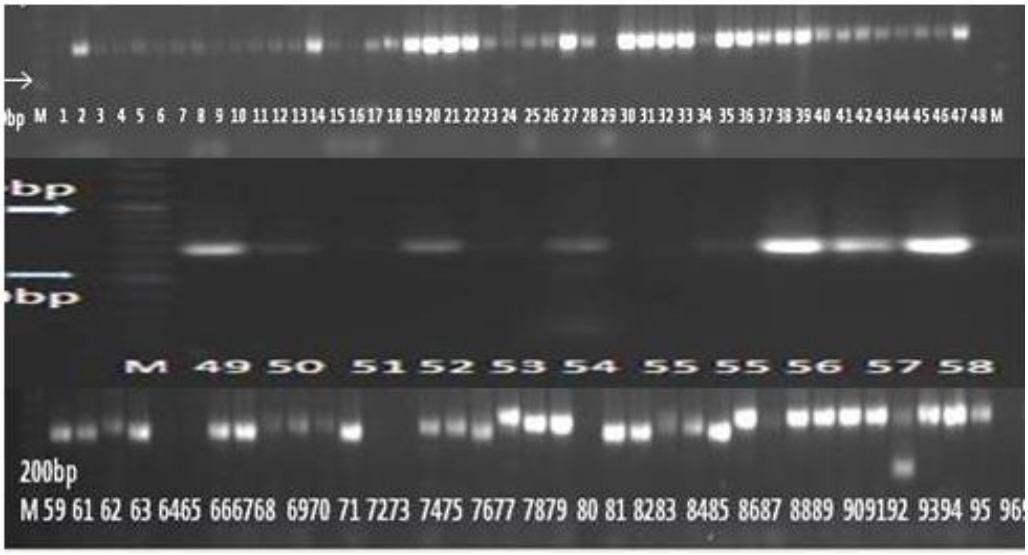


Fig. 34.DNA profile generated by SSR (**MB17**) primer ; M= marker-50bp; Lanes: 1, 44 and 47 (Shambat isolates); 2, 5-7, 13, 16, 18-20, 22-24, 28, 29, 36-38, 41, 42, 45, 46, 48, 51, 57, 58 (NewHalfa isolates); 3, 4, 8-12, 14, 15, 21, 26, 27, 30-35, 39, 40, 49, 50, 52-54, 59, 61, 63-70, 72, 77-79, 81-82, 85 (Gezira isolates); 17, 25 (Sennar isolates); 43, 55, 56, 60 (Hudeiba isolates); 62, 71, 73, 86-89 (Terbol, Lebanon); 74-76, 83, 84 (Burgeig and Bushariya isolates); 80 Barbar isolate; 90-96 (ICARDA sick plot, Syria)

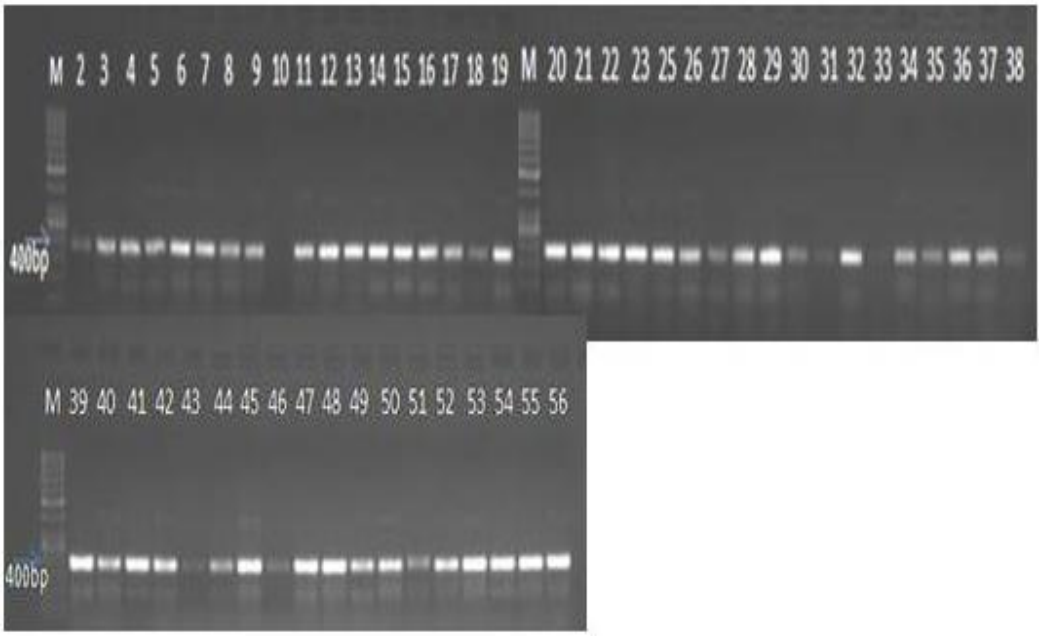


Fig. 35.DNA profile generated by SSR (**MB14)** primer; M= marker-1 kb; Lanes: 1, 44 and 47 (Shambat isolates); 2, 5-7, 13, 16, 18-20, 22-24, 28, 29, 36-38, 41, 42, 45, 46, 48, 51, 57, 58 (NewHalfa isolates); 3, 4, 8-12, 14, 15, 21, 26, 27, 30-35, 39, 40, 49, 50, 52-54, 59, 61, 63-70, 72, 77-79, 81-82, 85 (Gezira isolates); 17, 25 (Sennar isolates); 43, 55, 56, 60 (Hudeiba isolates); 62, 71, 73, 86-89 (Terbol, Lebanon); 74-76, 83, 84 (Burgeig and Bushariya isolates); 80 Barbar isolate; 90-96 (ICARDA sick plot, Syria)

Table 18. Total number of bands generated by SSR primers

Primer type	Primer name	Total number of bands	Amplicon size (bp)
SSR	MB14	3	400-600
	MB17	3	250-300
	MB18	5	250-900

4.3.4. Sequence characterized amplified region (SCARs)

SCAR primers developed from RAPD markers (Jiménez-Gasco and Jiménez-Diaz (2003) identify *F. oxysporum* f. sp. *ciceris* races 0, 1B/C, 6, 5 and 1A.

4.3.4.1. FocR0 M15 primer specific for race 0

This primer amplified only a single 900bp (Jiménez-Gasco and Jiménez-Díaz, 2003) product or bands from the genomic DNA of 58 Sudanese isolates which represent 23 isolates from New Halfa, Kassala State; 24 isolates from central and southern parts of Gezira State; 3 isolates from Hudeiba, River Nile State; 2 isolates from Shambat, Khartoum State and 2 isolates from Sennar State (Fig. 36). This indicates that these 58 isoaltes belong to Foc race 0 (groupA). However products from the isolates obtained from the Northern State, northern locations of Gezira State, Lebanon and Syria were not amplified by this specific primer.

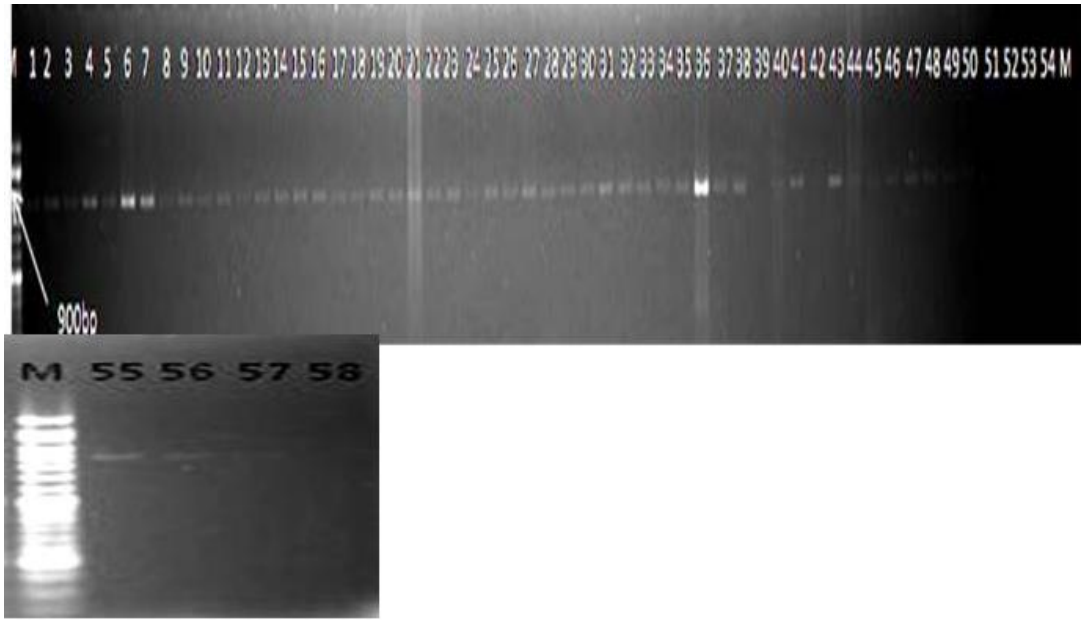


Fig. 36.DNA profile generated by **FocR0-M15**primer; M= marker-50bp Lanes: 1, 44 and 47 (Shambat isolates); 2, 5-7, 13, 16, 18-20, 22-24, 28, 29, 36-38, 41, 42, 45, 46, 48, 51(NewHalfa isolates); 3, 4, 8-12, 14, 15, 21, 26, 27, 30-35, 39, 40, 49, 50, 52-54, (Gezira isolates); 17, 25 (Sennar isolates); 43 (Hudeiba isolates).

4.3.4.2. FocR1B/CN5 primer specific for race 1B/C

This specific primer amplifiedabout 553bp (Jiménez-Gasco and Jiménez-Díaz 2003).DNA bands producing a profile for 7 isolates collected from ICARDA sick plot, Syria. No band was amplifiedin any of the Sudanese and Lebanese isolates (Fig. 37). This clearly indicated that the Syrian isolates belong to race 1B/C and non of the other isolates belong to this race.

Fig. 37.DNA profile generated by FocR1B/C-N5primer specific for race 1B/C with Syrian isolates sick plot. M= marker 50bp; Lane 90-96(Syria SP1-SP7).

4.3.4.3. FocR6-O2 primer specific for race 6

This primer is spesific for race 6 where the amplicon size is 1000bp(Jiménez-Gasco and Jiménez-Díaz, 2003). This primer did not amplify DNA fragment of the Sudanese and Syrian isolates. The primer only amplified bands around 1000bp diagnostic for Lebanese isolates collected from Terbol area (Fig. 38).This indicated that non of the Sudanese isolates belong to this race.

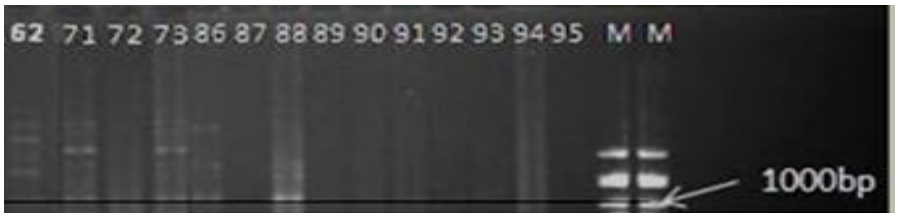


Fig. 38.DNA profile generated by FocR6-O2primer specific for race 6 with Lebanese isolates, M= marker- 50bp;Lane:62 (Terbol 1), Lane: 71-73(Terbo1-3), Lane: 86-88(Terbol 4-7).

4.3.4.4. **FocR5-L10** and**FocR6P18f/FocR0-M15r** specific for races 5 and 1A

These primers did not amplify any genomic DNA bands of all tested isolates from Sudan, Syria and Lebanon.

4.3.5. Gene specific marker Hop78F2/R2, DstF/R and XylF/R to identify races1A, 2, 3 and 4

The Hop78F2/R2, DstF/R and XylF/R primers were used to detect the Indian races 1, 2, 3 and 4 (Gurjar *et al.* 2009). In this study, the Hop 78F2/R2 primer amplified 1500 bp (Gurjar *et al.* 2009) product with 12 isolates fromthe isolates of Northern State,Barbar and Hudeiba Research Farm (River Nile State) and Aldibeba,Masalamiya, Wadmedani(GH) and Kaburfrom northern and central parts of Gezira State (Fig. 39A). No amplified DNA bands were detected in all isolates when DstF/R primer used and this indicated the absence of race 3 (Fig. 39B). XylF/R primer amplified 700bp products with the same isolates detected by Hop78F2/R2 primer and this indicates the absence of race 4 (Fig. 39C) among these isolates. Race 1Awas excluded when primer FocR6P18f/FocR0-M15r was used. This clearly support that these 14 isolates accommodated in group B belong to race 2.

None of the primers used was able to amplify DNA bands with any of the remaining 8 isolates. These isolates belong to group C and were collected from Gezira State only. These findings suggested that these isolates do not belong to races 0, 1A, 1B/C, 2, 3, 4, 5 and 6. Therefore, these isolates remain unidentified.

Fig. 39. Representative DNA profile generated by A **Hop78F2/R2,B Dst F/R and C Xyl F/R** primers and identified race 2. Lane 60:Hudeiba(48), lane68:GH(ARC), lanes 74-76 Burgeig, lane77: Kabur, Lanes 78-79: Masalamiya, Lane80:Barbar, lanes81,82,85:Dibeba, lanes 83-84 Albushariya

Table19.Identified races ofthe studied *Fusarium oxysporum* f. sp. *ciceris* isolates

No	Isolate name	Race	No	Isolate name	Race	No	Isolate name	Race
1	**Shambat 1**	**0**	41	New Halfa -18	0	81	Aldibebah1	2
2	New Halfa1	0	42	New Halfa 19	0	82	Aldibebah2	2
3	Dar Essalam 1	0	43	Hu.deiba 1	0	83	Albushariyah2	2
4	Um Trebat	0	44	Shambat 2	0	84	Albushariyah3	2
5	New Halfa 2	0	45	New Halfa 20	0	85	Aldibebah3	2
6	New Halfa 3	0	46	New Halfa 21	0	86	Lebanon 4	6
7	New Halfa 4	0	47	Shambat 3	0	87	Lebanon 5	6
8	Hilat Farah 1	0	48	New Halfa 22	0	88	Lebanon 6	6
9	Wad Haggar	0	49	Wad Haggar	0	89	Lebanon 7	6
10	Hilat Farah 2	0	50	Portubail 3	0	90	Syria1	1B/C
11	Wd Elabyadani	0	**51**	**New Halfa 23**	**0**	91	Syria 2	1B/C
12	Portubail 1	0	52	Hillat Farah 5	0	92	Syria 3	1B/C
13	New Halfa 5	0	53	Hillat Farah 6	0	93	Syria 4	1B/C
14	Hilat Farah 3	0	**54**	**Um Jeer 2**	**0**	94	Syria 5	1B/C
15	Alkumur 1	0	55	Hudeiba 2	0	95	Syria 6	1B/C
16	New Halfa 6	0	56	Hudeiba 3	0	96	Syria 7	1B/C
17	Sennar 1	0	57	New Halfa 24	0			
18	New Halfa 7	0	**58**	**New Halfa 25**	**0**			
19	New Halfa 8	0	**59**	**Wad Madani GRF)1**	**X**			
20	New Halfa 9	0	**60**	**Hudeiba 4**	**2**			
21	Um Jeer 1	0	61	Wad Medani (GRF)2	X			
22	New Halfa 10	0	62	Terbol 1	6			
23	New Halfa 11	0	63	Bagadi 1	X			
24	New Halfa 12	0	64	Almasalamiyah1	X			
25	Sennar 2	0	65	Bagadi 2	X			
26	Portubail 2	0	66	Wad Medani (GRF)3	X			
27	Portubail 3	0	67	Altalhah	X			
28	New Halfa 13	0	68	Wad Medani(GH)1	2			
29	New Halfa 14	0	69	Wad Medani (GH)2	X			
30	Dar Essalam 2	0	70	Wad Medani (GH)3	X			
31	El irayek	0	71	Terbol 2	6			
32	Alkumur 2	0	72	Wad Medani (GH)4	X			
33	Dar Essalam 3	0	73	Terbol 3	6			
34	Altalbab	0	74	Alburgaig1	2			
35	Um Tarfaya	0	75	Alburgaig2	2			
36	New Halfa 15	0	76	Alburgaig3	2			
37	New Halfa 16	0	77	Alkabur1	2			
38	New Halfa 17	0	78	Almasalamiya2	2			
39	Hillat Farah 4	0	79	Almasalamiya3	2			
40	Mahala	0	80	Barbar	2			

Shambat,New Halfa 23 and 25, UmJeer2, Wad MedaniGRF1 and Hudeib4 are the missed isolates

4.4. Primers data analysis

Only 90 isolates out of the 96 isolates tested were chosen for molecular analysis due to missing data or shortage of the tested DNA of these 6 missed isolates (Table 19).

UPGMA of the banding pattern grouped the 90 isolates into 2 main clusters at dissimilarity values ranged from 1 to 0.1(Fig.40)

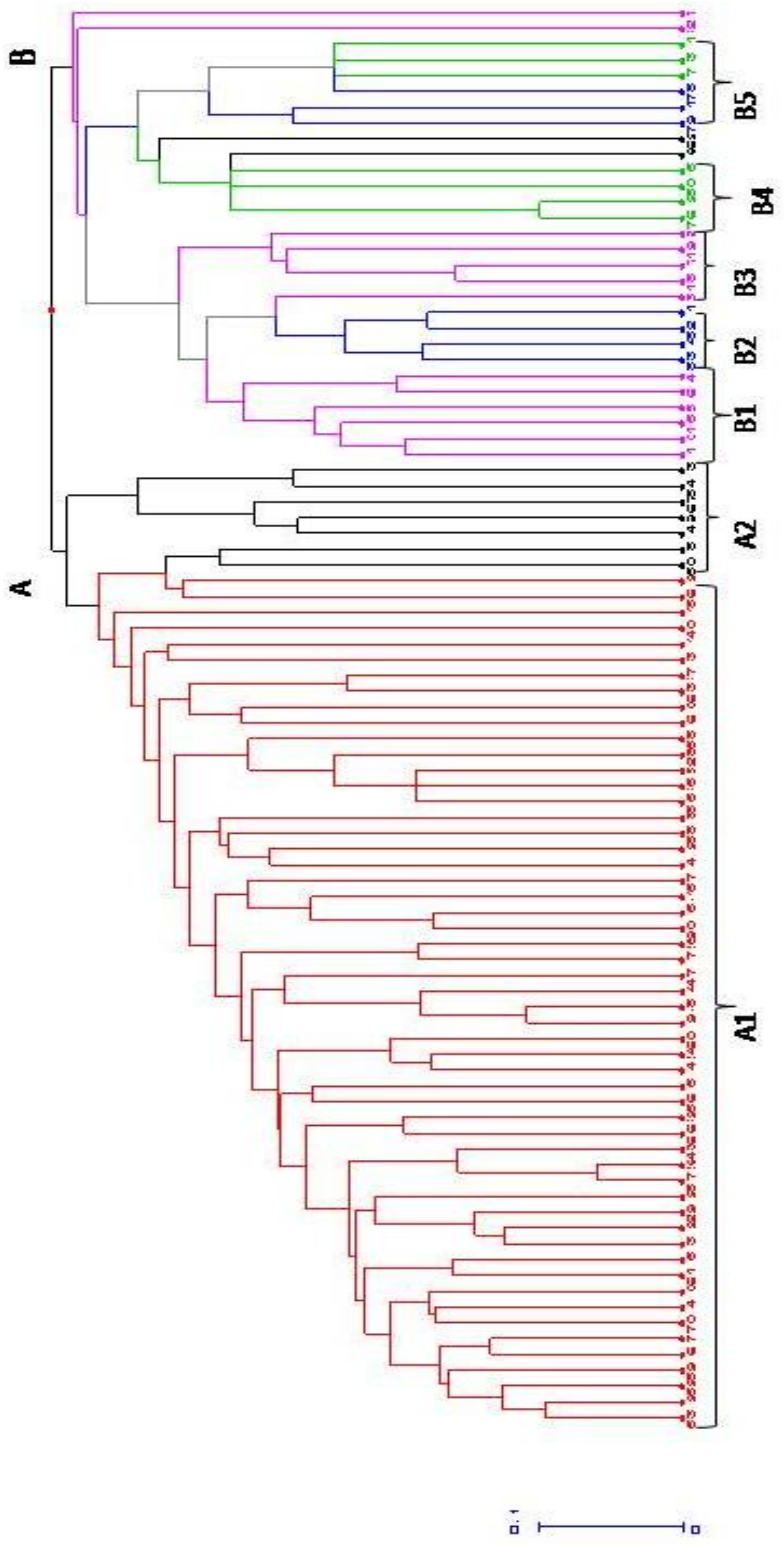


Fig. 40. Dendrogram derived from polymorphic DNA analysis of 90 isolates of *Fusarium oxysporum* f.sp. *ciceris* with 4 (RAPD) and 3SSRs primers (UPGMA).

4.4.1. Group A isolates

The isolates of this group are divided into 2 clusters (A1 and A2). The 54 isolates that constituted subgroup A1 are mainly from New Halfa (Kassala State), central and southernparts of Gezira State, Sennar, Shambat and Hudeiba (wilt and root rot plot). Whereas group A2 included three isolates from Wad Medani glasshouse (GH), two isolates from Bagadi, one from Almasalamiya and one from Altalha areas of southern and central Gezira State (Fig.40).

4.4.2. Group B isolates

This group accommodated five subgroups that contained the Syrian isolates from ICARDA sick plot and Terbol, in addition to isolates from northern and central Gezira, River Nile and Northern State of Sudan (Fig.40). B1 accommodated six isolates from Aldibeba, Alkabur, Almasalamiya and Barbar while B2 group contains four isolates three from Terbol of Lebanaon and one from Syria. B3 are three isolates from Alburgaig and two isolates from Albushariya areas of the Northern State. B4 group contains four isolates from Syria sick plot, while B5 includes three isolates from Terbol and three isolates from Syria.

4.5. Analysis of races

Cluster A1 isolates amplified 900bp products or bands from their genomic DNA with *Foc* R0 primer verified that they belong to race 0 (Fig.41). In cluster A2, the genomic DNA of these isolates were not amplified by any of the race specific primers used. Therefore, the race of these isolates is different from the 8 international known races.

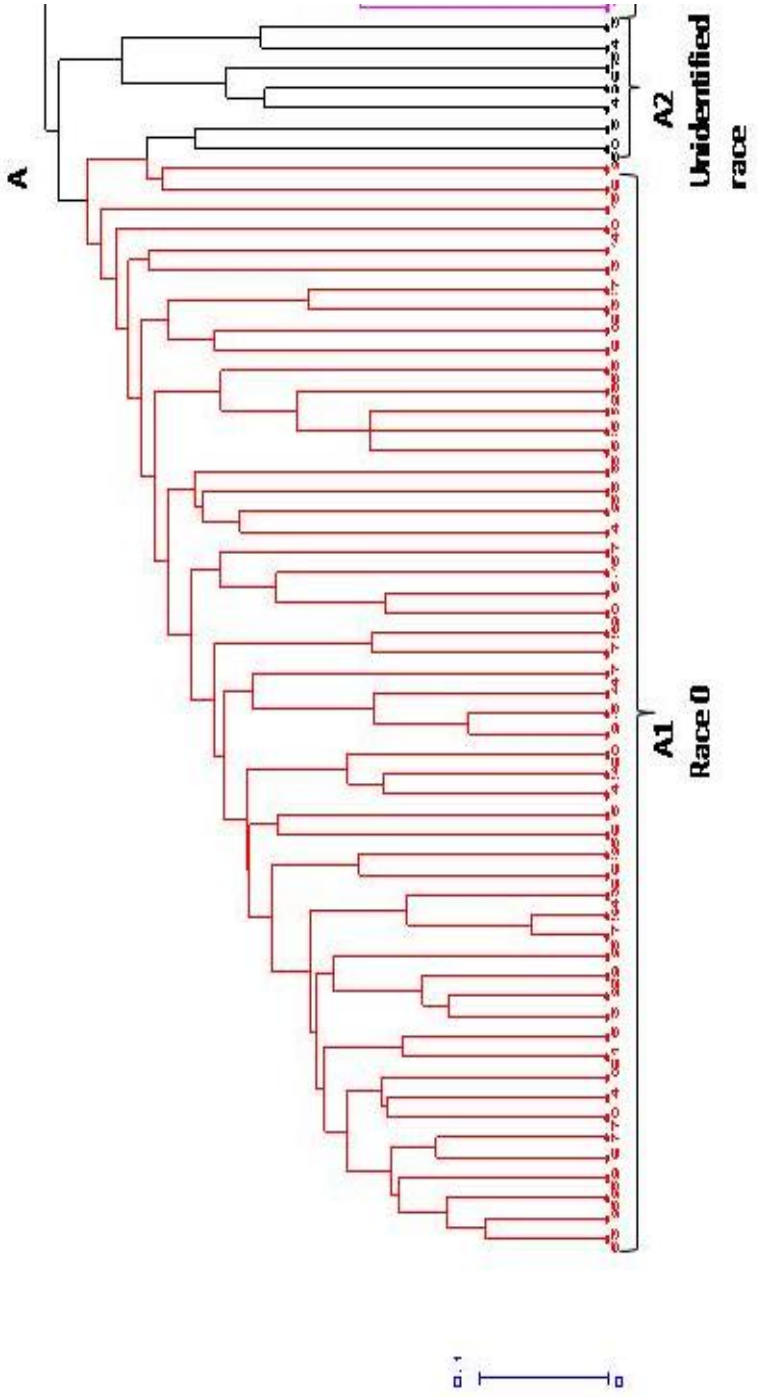


Fig. 41. Group A isolates with Race 0 and unidentified race

The genomic DNA of B1 and B3 clusters isolates amplified 1500bp bands with Hop78F2/R2 primer and were identified as race 2 (Fig. 42).

Clusters B2, B4 and B5 included the isolates from Syria and Lebanon which were included for comparison purposes and they were identified as race 6 for the Lebanese isolates and 1B/C for the Syrian (Fig.42).

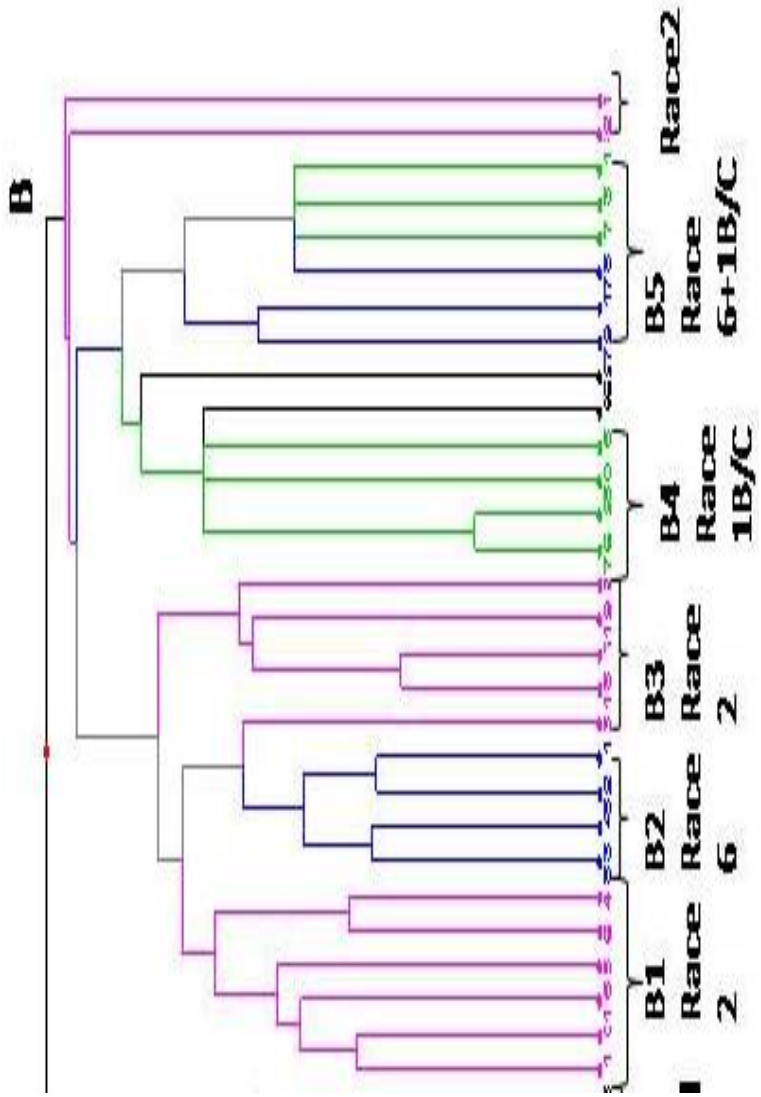


Fig. 42. Group B isolates with Races 2, 6 and 1B/C

4.6. Factorial analysis of all *Foc* isolates

The results of the factorial analysis placed the isolates of race 0 and the unidentified isolates in one group (A) and the isolates of races 6, 1B/C and 2 in the second group (B) (Fig. 43)

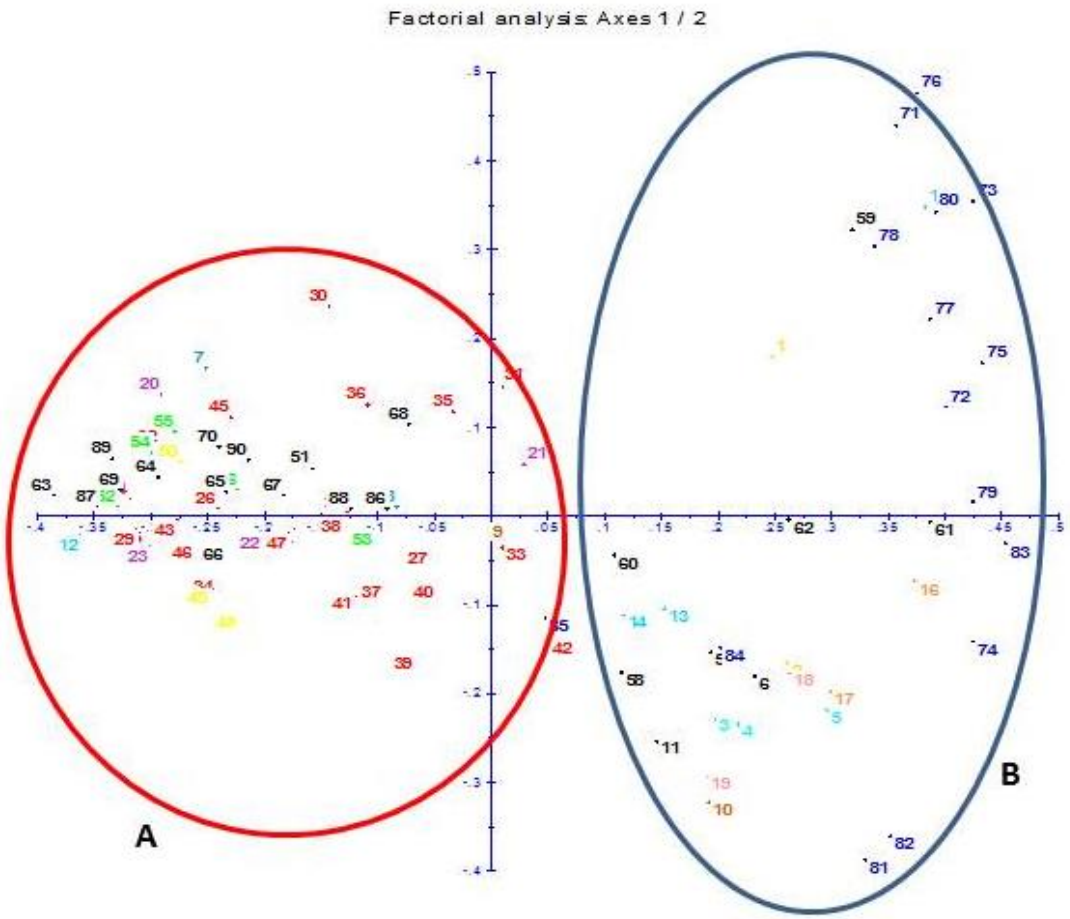


Fig. 43. Factorial analysis of all *Foc* isolates(A)race 0 + the unidetified race and(B) race 2 + 6 +1B/C

4.7. RAPD primers analysis

The bands of RAPD data were scored as binary digit code of "0" (for absence) and "1" (for presence), respectively. Analysis of binary data for all primers for the genetic similarity between pairs was estimated using Jaccard`s similarity coefficient which were used for the construction of UPGMA dendrogram as shown in Fig. 44. Cluster A contains some isolates from New Halfa, central Gezira, Sennar and Shambat sik plot. Cluster B includes Syrian and Lebanese isolates, whereas cluster C contains Alburgeig, Almasalamiya and Alkabur isolates. Albushariya and Aldibeiba isolates in cluster D. The isolates from Altalha and Glasshouse (GH, ARC) in cluster E and cluster F includes some isolates from New Halfa and central Gezira. Cluster G contains isolates from New Halfa, Hudeiba and central Gezira.

It is clear that race 0 is found in Gezira, Sennar, Kassala, Khartoum and River Nile States. Race 2 is found in Gezira and Northern State where the unidentified race is found only in Gezira state (Fig. 45)

Races distribution of *Foc* isolates collected from Sudan (Fig. 45) Showed that rcae 0 was prevalent in Gezira, Kasala, Sennar and River Nile States whereas race 2 was mainly found in Northern State and north of Gezira State. The unidentified race was only found in central Gezira.

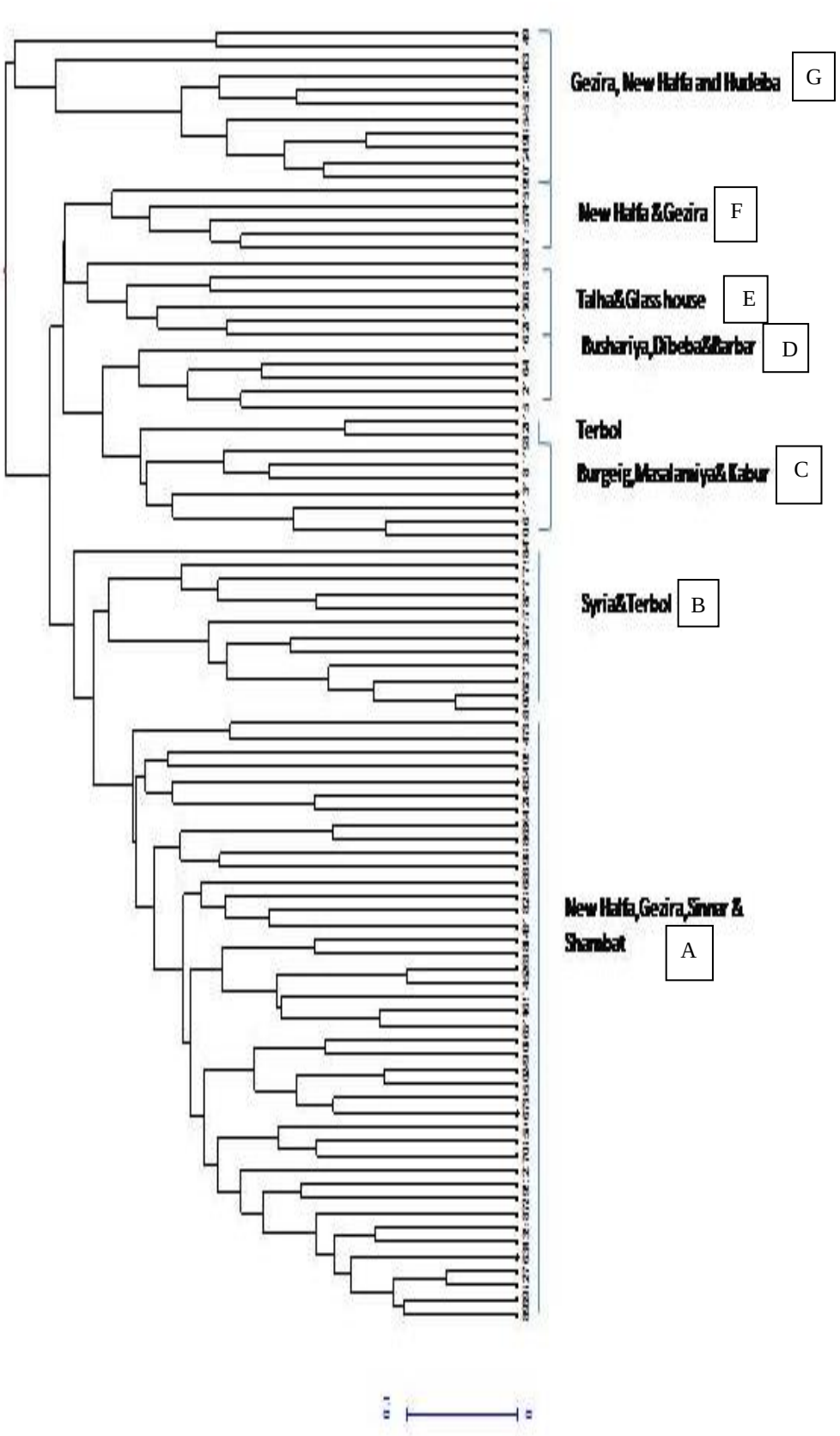


Fig. 44. Dendrogram of RAPD data

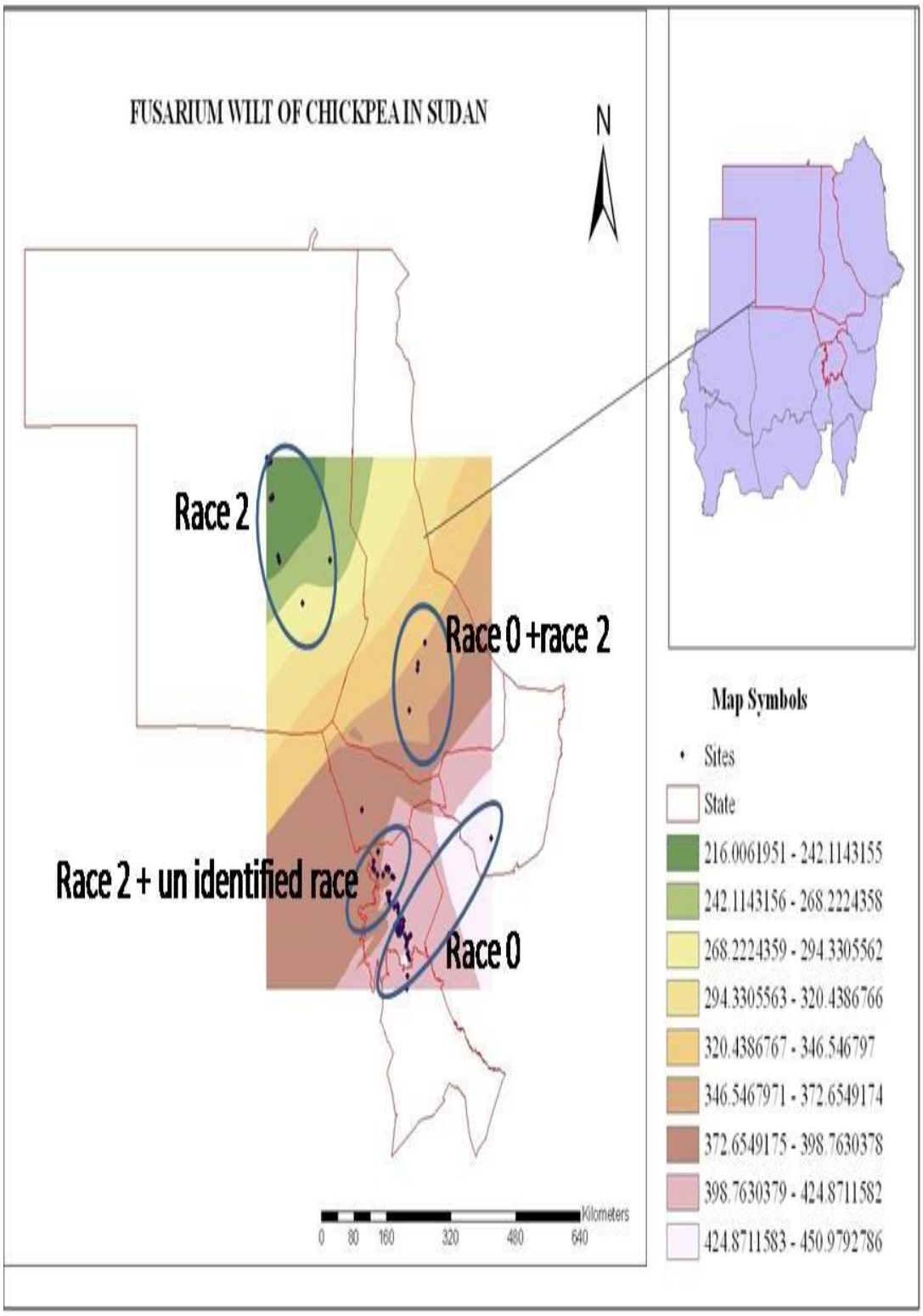


Fig. 45. Sudan map showing geographical locations and race distribution of *Fusarium oxysporum* f. sp.*ciceris*

4.2.4. Screening of chickpea germplasm to wilt resistance

According to the molecular characterization, three races of *Foc* were identified in Sudanese isolates. These races were 0, 2 and unidentified race (X).Twenty chickpea germplasm were examined for their reaction to these three races. Two isolates representing each race were used. The three races showed variable virulence on the chickpea cultivars and lines. Race 0 isolates showed progressive yellowing of the plants and wilting within 45 days after inoculation, whereas race 2 isolates exhibited wilting of the plants after one month from inoculation. Race X (unidentified) showed wilting of the plants within 45 days after inoculation without yellowing. Jebel Marra, Shendi, Elixir and Flip84-48c were the only genotypes susceptible to race 0. All other genotypes were either resistant or moderately resistant to the same race. All genotypes except Hawata were susceptible to the unidentified race (X). However, Jebel Marra and Shendi were highly susceptible to this race.The cultivars Jebel Marra, Shendi, Wad Hamid, Salawa, Matama, ILC464, FLIP 84-79C, FLIP03-104c and FLIP 84-48c were highly susceptible to isolates of race 2. Hawata reaction to the 3 tested races was either resistant or moderately resistant (Table 20).

Table 20. Reaction of chickpea germplasm to Race0, Race2 and the unidentified race of *Fusarium oxysporum* f. sp. *ciceris*

No.	Germplasm	Reaction to race 0	Reaction to race X	Reaction to race 2
1	JebelMarra (ILC 915)	S	HS	HS
2	Shendi (ILC 1335)	S	HS	HS
3	Hawata (Iccv-92318)	**MR**	**R**	**MR**
4	Wad Hamid (Iccv-2)	R	S	HS
5	Burgaig (Iccv-91302)	R	S	R
6	Salawa (Flipc82-89)	R	S	HS
7	Matama (Flip 77-91c)	R	S	HS
8	Atmour (Iccv-89509)	MR	S	HS
9	Elixir	S	S	R
10	Flip93-93	MR	S	R
11	ILC 1929	R	S	R
12	Flip97-263c	MR	S	R
13	ILC 464	MR	S	HS
14	Flip 84-79c	MR	S	HS
15	Flip 81-71c	MR	S	R
16	Flip 97-530	R	S	R
17	Flip03-104c	R	S	HS
18	ILC 3279	R	S	R
19	Flip84-48c	S	S	HS
20	Flip 85-17c	R	S	R

R=0-10% wilted plants, MR=21-40%wilted plants, S=41-80% wilted plants and HS=≥80% wilted plants

CHAPTER FIVE
5. DISCUSSION

Chickpea (*Cicer arietinum* L) is one of the most important food legumes in Sudan. The area under chickpea is annually increasing with the increase in demand, as well as the extraordinary higher prices of chickpea. Chickpea could possibly elevate the living standars of some farmers. The crop is suffering from some important diseases among which Fusarium wilt caused by the fungus *Fusarium oxysporum* f. sp. *ciceris* is reported to cause economic yield losses and in severe epidemics it may result in total crop losses (Ali *et al.,* 2002).

5.1. Disease survey

The survey conducted during thisstudy revealed that Fusarium wilt is one of the most limiting factors to chickpea production especially in the traditional areas in north Sudan. Chickpea production in Sudan was extended to new areas in the central part of the country (Gezira State). The cultivated areas were increasing annually since the introduction of the crop in early 90s (FAOSTAT, 2012).

More than 70% of the farmers grow "Baladi" variety which is highly susceptible to the disease, while less than 30% grow the released less susceptible cultivars e.g. Wad Hamid, Atmour (Ali *et al.,* 2002). This was obviously due to unavailability of released cultivars' seeds. The continuous cultivation of "Baladi" variety and exchange of seeds between farmers had led to rapid spread of the disease to new areas and speedy epidemics to Gezira State. *Fusarium oxysporum* f. sp.*ciceris* is a soil-borne pathogen and known to survive in soil for up to six years (Singh *et al.,* 2007).This has clearly resulted in high disease incidence in new areas even when farmers grow chickpea in sorghum, wheat and cotton series. The highest

disease incidence (42%) was found in monocroping systemwhen chickpea followed chickpea in the rotation. Yadav *et al.* (2007), reported similar results and concluded that cultivation of chickpea in the same area enhance buildup and accumulation of the pathogen. About 40% of chickpea farmersexperienced low disease incidence when chickpea was preceded by cereals in their fields. This practice was not supported by any research findings, but based on farmers' observation that gained interest of chickpea farmers.Gaur*et al.*(2010), reported that followinglong term crop rotations with cereals such as sorghum, wheat and milletwould help in managing the disease.

Chickpea farmers use a substantial wide range of seed rates (15-125kg/ha). The survey results clearly revealeda positive correlation between seed rates and percent disease incidence.To maximize chickpea yield and pest management, the crop should be planted on ridges at a seed rate of 60 kg seeds/ha (Ibrahim,1996). The seed rates used by farmers were either far below or above the recommended rates. The higher seed rates result in higher population densities and hencehigh competition between plants for nutrients and water.Consequently, the plants become very weak and vulnerable to invasion by wilting pathogens. In previous studies,wilt disease of leguminous crops (faba bean) was managed by late sowings. The reduction in disease was incidence correlated well with the decrease in temperature (Freigoun, 1980b; Salih and Ageeb, 1987; Ageeb *et al.*,1989; Ibrahim and Ali, 1993; Ali, 1996). Sheikh Mohamed and Van Rheenen (1991) considered mid Novemberoptimumfor crop yield and disease management. However, recently with the global climate changes and fluctuations of tempertatures between seasons, it became difficult to stick to this recommended sowing dates for disease management. During the survey, we found that only 15% of chickpea farmers were sticking to mid November sowings and they experienced high disease incidence of 31%

compared to 5% in late sowings of December. According to weather speculations, chickpea farmers shifted sowing date from early to late December to escape the high temperatures and consequently high disease incidence. By doing so, they were confronted by low crop yields.In this study, we found that disease incidence was highly corelated to sowing dates. Late sowings of chickpea exhibited lower disease incidence as the temperatures started to decrease. The early sowings of the crop were subjected to temperatures higher than 25˚C of early winter which favour chickpea pests and diseases. Our observation was supported by published data. Landa (2006) reported that the resistance in some chickpea cultivars to Fusarium wilt may be temperature-dependent and warmer temperatures associated with later sowings may affect the disease reaction of these cultivars.

Several studies have suggested that higherdisease control and yield are obtained when theplanting is delayed until the last week of October (Chand and Khirbat, 2009). The lower diseaseincidence in late-sown crop was considereddue to low temperature prevailing during the periodof late-sown crop. The studies ofNavas-Cortes *etal.* (1998) showed that for each year of experimentepidemic development was related mainly to thedate of sowing. Chand and Khirbat (2009) reported that advancing the sowing date for chickpea crop in southern Spain from early spring to early winter could slow down thedevelopment of wilt epidemic, delay the epidemic onsetand minimise the final amount of the disease.

Watering intervals for the crop is an important factor that affects disease incidence. Previous studies proved that increasing the gap between irrigations resulted in increasing disease incidence (Ali, 1996). Findings of this this survey showed that frequent irrigation of1-week interval perform better than 2 or 3-week intervals for management of the disease. However, >65% of the farmers adopted 2-3 weeks watering interval, while only 10%

of them water their crop every week. There was an indication that irrigation interval play an important role in disease prevalence and incidence. However, it is difficult for chickpea farmers to adopt calender based watering regime with the current situation of irrigation water fluctuation in the canals. In addition, a number of crops in the rotation are competing in irrigation water at the time of chickpea cultivation.The yields of leguminous crops are known to be sensitive to weather conditions, particularly temperature and relative humidity which is influenced by irrigation intervals. Chickpea yield is known to be reduced by high temperatures and over and under watering (Ageeb, 1976; Freigoun, 1980b; Ali, 1996; Landa*et al.*, 2006)

The questionnaire results indicated that all farmers were not using seed dressings to protect chickpea seedlings from diseases and in particular Fusarium wilt. In Sudan, use of fungicides seed dressings was reported to delay the onset of chickpea wilt compared to untreated seeds (Ali *et al.*, 2002; Mahir *et al.*, 2007; Hamed, 2012) but not significantly reduced the final disease incidence. However, Ali *et al* (2002) found that the fungicide Tecto -TM and Quinolate pro increased seedling emergence in the wilt infested plot. Variability in the response of *Foc* to several fungicides, *in vitro*, was reported by several authers (Christian*et al.*, 2007; Singh and Jha, 2003; Ayyub, 2001). Seed dressings could be an important component of disease management and espcially needed to eradicate seed borne inoculums,increase the plant vigour and enhance good crop establishment.

In this study, we found that 50% of farmers do not apply fertilizers to their crops. As chickpeais a leguminous crop capable of acquiring a large portion of the nitrogen (N) it needs from the atmosphere and form nodules on theroots, applying fertilizers to the crop is not expected to have significance in reducing the disease incidence.

The control measures against seed and soil-borne Fusarium wilt of chickpea like any other crops may include the use of disease free seeds, seed dressing with appropriate fungicides and use of resistant cultivars. Crop rotation, particularly in case of this disease is not effective since the pathogen can persist in the soil for years. This fact was well noticed by many researchers (Haware *et al* 1992; Singh *et al.*, 2007).

The average disease incidence inthe heavy black clay soils of south Gezira was the highest (27%) among the surveyed areas. In Northern State, although the cultivated areas were small, disease incidence (21%) was higher than in River Nile state (12%) with sandy clay soils. The least disease incidence was observed in the light clay soils of north Gezira (11%). The results of the survey indicated that light clay and sandy clay soils exhibited lower disease incidence as compared to heavy clay soils. This is apparently due to large particles and pore spaces whichallow water to drain quickly and easily through sandy andlight soils. Due to this simple fact sandy soils drain quickly and do not hold large amount of water. Clay soils consisted of very fine microscopic particles that fit together tightly resulting in tiny pore spaces. The tiny pore spaces allow water to move through them, but at a much slower pace than in sandy soils. Clay soils drain quite slowly and hold more water than sandy soils and this soil moisture harbor so many soil pathogens including chickpea wilt pathogens. The results clarified that the soil type and temperature are known to influence chickpea wilt disease development. Drought and high soil temperature were found conducive to chickpea wilt and previous studies clearly distinguished the significant effects of environmental and soil conditions on chickpea wilt disease incidence development (Sugha *et al.*, 1994b; Khilare and Rafi, 2012Mehmood*et al.*, 2013). At the same time, chickpea is very sensitive to high soil water contents.

The heavy black soils of southern Gezira exhibited 27% disease incidence and it was the highest among Sudan states.Thisfinding is supported byChand *et al* (2009) who reported that in some studies done black soilswere found to support highest wilt incidence than in sandy-loam, red and clay soils. Other studies substantiated the fact that the disease is more severe in light sandy soil than heavy clay ones because of their low water retention ability (Sugha *et al*, 1994b). The variation in results, support that chickpea wilt disease development or progression is affected by many biotic and abiotic components and it is difficult to relate any single factor to disease management. This study verified that Fusarium wilt of chickpea in Sudan was adverslyaffected by natural factors whichwere further magnified by the use of traditional low yielding cultivars and the poor management practices.

5.2. Variability among*Fusarium oxysporum* f. sp. *ciceris* isolates

Differences in morphological characters of the isolates on PDA media were observed when cultures were incubated at 25˚C±2 for 7 days. The isolates were clustered according to colony diameter, colony texture, pigmentation and micro and macroconidia sizes into three clusters. The isolates of the first cluster (A), represented 71% of the isolates,were fast growing with white pinkish feathery growth. The second cluster (B), represented 17% of the isolates were very slow growing, with white pinkish cottony growth. The third cluster (C), represented only by 12% of the isolates and were intermideate in growth with white pinkish mycelia which ultimately developed into violet or yellow pigmentation. The Syrian (group D) and Lebanese (groupE) isolates were fast and intermediate growing, respectively. The Syrian isolates with whitish feathery mycelia that developed into dark violet pigmention, while the Lebanese with whitish cottony mycelia that developed into orange pigmentation.

Fusarium oxysporum has been reported to develop pigmentation on PDA growth medium (Joshi*et al*. 2012). The aerial myceliumof the fungus is usually white and can change to a variety of colors fromlight purple to dark purple, pink and orange depending on the strain of *F.oxysporum*. Morphological variabilityof *Foc* was well documented. Arvayo-Ortiz*et al* (2011) detected a high degree of phenotypic and genomic variability in the strains of *Foc* in Mexico. Joshi *et al*. (2012) compared colonies of *Foc* race 2 with colonies of Foc race 0 and found that race 2 colonies, produced a dense white, aerial mycelium that was evenly spread on the growth medium,whereas race 0 colonies produced a white aerial mycelium, which was not as dense and was less even on the surface of the plate. It hasalso been suggested that growth conditions such as medium, light and temperature can encourage pigment production in *F. oxysporum* (Rodrigues *et al.,* 2005).

Differences between *Foc* isolates in number and sizes of micro and macroconidia were observed in this study. Micro and macroconidia were abundant in clusters B and C. However, the macro conidia in the cluster Awere wider than in B and C clusters.The third cluster (C) exhibited the longest macroconidia among all groups.Similar studies had been reported by Arvayo-Ortiz*et al.* (2011), Sharma *et al.*(2009)and Honnareddy *et al.* (2007). Generally, the microconidia of all isolates were 0-1 septate, while macroconidia were 2-4 septate. Clamydospores were observed only in cluster A and the Syrian isolates.

5.3. Pathogenic variability

In this study,only nine chickpea differentials of the set developed by Haware and Nene (1982)wereavailable and used to distinguish pathogenic variability among isolates collected from different states in Sudan. Unfortunately, the test was only performed for 25 isolates due to

unavailability of enough seeds of the differentials. All isolates tested showed progressive yellowing of leaves and then complete wilting of the plants. The differentials showed resistant reaction to all tested isolates and this confirms that these isolates belong to race 0 (Halila and Strange, 1997). Race 0 was foundnot pathogenic to chickpea cultivar JG 62 but pathogenic to kabuli chickpeas (Jim´enez-D´ıaz *et al.*, 1993a). This may explain the susceptibility of the kabuli type cultivar Shendi to this race. Desi chickpeas are grownprimarily in the Indian subcontinent and most of them are resistant to race 0 which explains the abscence of race 0 in India. Although the usefulness of this method for identification of pathogenic variabilityis not questionable, it requires a long time to assess variability. At the same time plant reaction to the pathogen could be influenced by different environmental parameters, as well as conditions during which the test was performed. These constraints were well highlighted by Haware and Nene (1982). In addition,seeds of the assigned differential cultivars are not always available.Their development and maintainance is costly and time consuming. In addition, evaluation of the reaction of the differentials to the pathogen could be possibly subjected to human errors. Sharma *et al.*(2005) reported that monitoring reaction of plants to *Foc* is different from one person to another.

DNA-based techniques have increasingly become the tool of choice for understanding the genetic diversity of Fusarium species. Therefore, there is a need for new, consistent, improved methods for the rapid, reliable, and reproducible identification and quantification of *F. oxysporum* f. sp. *ciceris* races in Sudan.

5.4. Genetic variability and race identification

This is the first study for molecular characterization of genetic diversity and race identification of Fusarium wilt causing pathogen (*Foc*) in major chickpea growing areas in Sudan. Studying the variability of *Foc* from various agro-climatic zones in Sudan is not only important for disease management but also necessary for the selection of resistant chickpea cultivars.

The primers used in this study FOCP1 and FOCP2 demonstrated that the two primer sets are reliable and can be used to assess pathogenicity tests for *F. oxysporum* f. sp. *ciceris*and findwhether they are pathogenic or not. They can also be used todifferentiate *Foc* from speices and other formae specials of *F. oxysporum* (Jiménez-Fernández*et al.*, 2011).

Variabilty among isolates at DNA level is expected to to be more reliable as it is not influenced by environmental changes.The present study clearly characterized the isolates collected from different chickpea growing areas of Sudan into 3 groups or races of *Foc.*These are namely 0, 2 and unidentified race (X) that did not correspond to to any of the eight races reported worldwide.

Based on the reaction of chickpea differential varietiesto the tested *Foc* isolates, previous studies in Sudan reported prevalence of race 2 in the River Nile State. In addition, six unidentified races distinct from the eight internationally known races were also reported in River Nile and Khartoum States (Ali, 1995; Suliman, 2000; Kurmut, 2002). The unidentified races detected in this study could be similar or different from the unidentified racesreported by Ali (1995), Suliman (2000); Kurmut (2002). This will only be verified if we perform more pathogenicity tests using differential varieties in future.This necessitates that more work is needed to cover all

chickpea cultivation areas in Sudan to detectoccurence and distribution of other possible races.

The cluster analysis of the 90 isolates tested using four RAPD and three SSRprimers revealed that the Sudanese isolates are different from the Syrian and Lebanese isolates. This suggested that Sudanese isolates do not include race 6 and race 1B/C. It is evident that 71% of the Sudanese isolates belong to race 0, 17% belong to race 2, 12% to the unidentified race. Race 0 is widly distributed in central Sudan, while the unidentified race is limited to Gezira State.This clearly indicates that race 0 is the most prevalent and widley spread. Therefore, some measures should be taken to prevent its spread to other new areas. Race 2 is prevalent in Northern State, River Nile State and northern parts of Gezira State and was only detected in Glasshouse, ARC WadMedani.

Random Amplified Polymorphic DNA(RAPD) markers have the advantage of detecting polymorphism simply and quickly, while SimpleSequence Repeats (SSR) markers or microsatellites provide higher producibility and genetic informativeness. Both markershave been used in molecular characterization of *Foc* isolates (Dubey *et al*., 2012) as well as of other Fusarium species (Datta and Lal, 2013).

Recently SSR markers targeting resistance genes in chickpea for different races of *Fusarium oxysporum* f.sp.*ciceris* (*Foc*) have been identified (Ali *et al.*, 2012). These markers can effectively be used for screening local genotypes against Fusarium wilt disease and very useful in the development of Fusarium wilt resistant germplasm and breeding programs.These promising markers showed good correlation with phenotypic evaluation of genotypes to different races of *Foc*, except those markers for race 3 because they showed deviations from phenotypic data and the reason might be that race 3 as found by Gurjar*et al.* (2009) is actually *Fusarium proliferatum* and not *Fusarium oxysporum*. As this race

doesn’t belong to *Foc* anymore, resistance to it might involve some other major resistance genes.

The screening of Sudan chickpea germplasm against the identified races (0, 2 and unknown), demonstrated that the already released cultivars (Shendi and Jebel Marra) were susceptible to the 3 identified races of the pathogen. If we need to save Shendi and Jebel Marra as high yielding and good quality chickpea cultivars, incorporation of the resistant genes of Fusarium wilt pathogen to those cultivars becomes indispensable. Hawata variety is a promising variety resistant to the three races and could be introduced in demonstration fields to chickpea farmers. The released varieties Wad hamid, Burgaig, Salawa, Matama and Atmour have fluctuating reactions to the three races. All the tested cultivars and lines are susceptible to the unidentified race found in Sudan and this indicates that it is the most aggressive race in Sudan. To overcome this situation in areas where this race is prevailing, a large chickpea germplasm should be screened for resistance to this race. Similar results of variation in chickpea resistence were reported in Sudan, India, Pakistan, Italy, Syria and Tunisia (Ali *et al.,* 2002; Nene and Haware, 1980; Ahmad *et al*., 2010; Chaudhry *et al.,* 2006; Iqbal *et al.,* 2010; Chaudhry*et al.,* 2007; Infantino*et al.,* 2006; Iqbal *et al.,* 1993, Jim´enez-D´ıaz *et al.,* 1993b and Halila and Strange, 1997). Also the greenhouse screening of chickpea should be extended to field testing to confirm resistence in the field. Again the stage of plant growth at which infection occurs is crucial.

Conclusion

- From the questionnaire done with chickpea farmers, we can conclude that the traditional variety "Baladi"is the most susceptible variety to the disease, while the varieties Atmour and Wad Hamid exhibited lower disease incidence.
- Chickpea grown in heavy clay soils of Gezira exhibited higher disease incidence than that grown in sandy soils as in River Nile State. As the crop is newly introduced in Gezira and the area is annually increasing, a package of recommended cultural practices for chickpea farmers should be developed and released.
- In monocropping system the disease incidence is higher than the other preceding crops.
- According to the great changes in climatic conditions during winter season, the effect of sowing date on disease incidence needs to be studied precisely and the recommended sowing dateneeds to be revised as the late sowings in December exhibited lower disease incidence as comparedwith the early sowings in October and early November. Most farmers included in the survey tend to grow their crop on mid November.
- Since the higher seeded fields exhibited higher disease incidence,the higher seeding rates above 60kg/ha are to be avoided and seeding rate of 60kg chickpea seeds/ha should be used as recommended by ARC.
- Applying fertilizers to chickpea as far as this study is not important. However, its significance in reducing wilt disease incidence and improving chickpea yield need to be verified.

- The shorter irrigation intervals can reduce chickpea wilting but the amount of water per irrigation should be carefully monitored as the crop can notwithstand excessive water.
- The findings of this study clearly demonstrated the discriminatory supremacy of molecular techniques in the identification and separation of *Foc* isolates according to their race.
- Molecular markers (e.g. race-specific primers) are the most effective tools in characterization and race identification of *Fusarium oxysporum* f. sp.*ciceris* because they are precise,rapid and reliable in detection and identification, unaffected by environmental parameters, and safe a lot of time for researchers.
- Three races are found in Sudan, rcae 0 is prevalent in Gezira, Kassala, Sennar and River Nile States whereas race 2 was mainly found in Northern State and north of Gezira State. The unidentified race was only found in central Gezira.
- This study would be useful not only to design and develop effective management strategies for chickpea wilt disease but also helpful to breeders to design effective resistance breeding programs in chickpea.
- Chickpea germplasm screening done with the three races indentified in in this study revealed that the cultivars Shendi and Jebel Marra are highly susceptible to these three races, while Hawata variety showed resistant reaction to the same races.
- The effective method for controlling the disease remains to be through the use ofresistant cultivars**.**
- Chickpea breeding lines and cultivars should be thoroughly screened for resistance to Fusarium wilt disease before being released for commercial production.

- Certified seeds of the promising resistant cultivars should be produced and distributed to farmers to replace the "Baladi" and other wilt susceptible chickpea cultivars.

Recommendations

1/ Close monitoring of the disease needs to be continued across all chickpea cultivation areas especially the newly expanded areas of Gezira.**2/** It is recommended not to sow Shendi and Jebel Marra cultivars in areas with a history of high disease incidence.

3/ Recommended sowing datefor chickpea is to be revised due to climatic changes.

4/ The higher seeding rates above 60kg/ha are to be avoided

5/ A package of cultural practices for chickpea farmers should be devoloped and released.

6/ Certified seeds of the resistant cultivars like Hawata and Wad Hamid should be produced and distributed to farmers.

7/ Disease free seeds should be treated with fungicides before sowing to control seed and early seedling infection and enhance good crop establishment.

8/ Long term crop rotations with cereals such as wheat, sorghum and millets should be followed.

9/ Undecomposed debris should be removed from the field before sowing for effective integrated disease management programs.

10/ This work should be continued to cover all chickpea cultivation areas in Sudan to investigate occurence and distribution of other possible races.

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Publications

Mohamed O. E., Seid Ahmed Kemal, Aladdin Hamweih and Ahmed N. E (2015).**Genetic Variability of *Fusarium oxysporum* f. sp *ciceris* Population Affecting Chickpea in the Sudan**. Journal of Phytopathology (In press)

Mohamed O. E., Ahmed N. E and Seid Kemal Ahmed (2014).**Cultural and Morphological Variability in *Fusarium oxysporum* f. sp. *ciceris* Causing Wilt of Chickpea in the Sudan**. In :PostersAbstracts Book, The 3rd Conference of Pest Management. 3-4 February, 2014. Agricultural Research Corporation, Wad Medani, Sudan.61

Chickpea Fusarium Wilt survey Form

Surveyor name: _____

Date of survey (dd/mm/yy): _____ / _____ / _____

Site no. : _____

Location name: _____

Latitude (decimal degrees): N_____

Longitude (decimal degrees): E _____

Elevation: _____ meters

Field area (ha):_____**Soil Type**_____

Chickpea type: Kabuli_____ Desi _____

Variety: _____ Improved _____ Land race (Local) _____

Sowing date: _____ **Seeding Rate** (kg/ha) _____

Previous crop: _____ **Irrigation intervals**_____

Fertilizers: No/Yes_____ Type and Name_____

Rates and time of Application: _____

Pesticides: Seed dressing _____OR foliar spraying (How many) _____

Herbicide Application: Yes (Name) _____ No_____

Disease incidence(%) _____

Disease symptoms: _____

First symptoms: _____ Stage at first symptoms: _____

Remarks:

_____

_____

_____

_____

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