Sudan University of Science and Technology

College of Graduate Studies and Scientific Research

Effect of Bacteria (Bacillus subtilis) against Fungus (Fusarium oxysporum) on tomato

تاثير البكتيريا (باسيلس سبيتالس) ضد فطر(الفيوزيريم اوكسيسبوريم) في الطماطم

A thesis Submitted in Partial Fulfilment of the Requirements of the Degree for B.Sc. (Agric.) in Plant Protection

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بسم الله الرحمن الرحيم

قال تعالى:

(أَلَمْ تَرَ أَنَّ اللَّهَ أَنزَلَ مِنَ السَّمَاءِ مَاءً فَسَلَكَهُ يَنَابِيعَ فِي الْأَرْضِ ثُمَّ يُخْرِجُ بِهِ زَرْعًا مُّخْتَلِفًا أَلْوَانُهُ ثُمَّ يَهِيجُ فَتَرَاهُ مُصْفَرًّا ثُمَّ يَجْعَلُهُ حُطَامًا إِنَّ فِي ذَلِكَ لَذِكْرَى لِأُوْلِي الْأَلْبَابِ)

صدق الله العظيم

سورة الزمر (الايه ٢١)

Dedication

To my father…To my mother

To my Family…

To my teachers…

To all my frinds….

With respect

Hala

Aknowledgments

All thanks are due to al mighty Allah who gave me health and power and helped me to produce this work. I would like to express my thank to my supervisor DR. Ekhlass Hussein for helpful assistance guidance… Patience and keen supervise during this work. Also my thank to M.S. Mwda and all my friend …Grateful thanks are due to all the staff of department plant protection, college of agricultural study, Sudan University of science and technology…

Finally great thank are extended to all one who help my and support to me during this study…

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ABSTRCT

This study was carried out in the laboratory of plant pathology, plant protection Department, College of Agricultural Studies, Sudan University of Science and Technology in (2017) the objective of this study was to evaluate The efficacy of Bacillus *subtitles* and (Fulldazim 50 WP) fungicides against the growth of *Fusarium oxysporum f.s lycopersion* in tomato in culture media Potato dextrose agar (PDA) *in vitro*. The fungi are an important causing significant reduction in yield. In the present study, the pathogenic fungi were isolated from infected plant parts .The fungi were identified based on morphological and culture characters as *Fusarium oxysporum f.sp* Lycopersion. The *Bacillus subtitles* were used for antagonistic potentials against the fungus and they used (Fulldazim 50 WP) fungicides. The result showed that the best performance (100% inhibition) followed by *Bacillus subtitles*. No significant differences between the funguses grow in control and the fungus grown with the *Bacillus subtitles*.

ملخص الاطروحة

اجريت هذه الدراسه في معمل امراض النبات, قسم وقايه النبـات كليـة الدراسـات الزراعيـه جامعه السودان للعلوم والتكنولوجيا في اغسـطس – سـبتمبر٢٠١٧ وذلـك لدراسـة اثرقـوة التضاد ضد الفطر. الهدف من هـذة الدراسـة تقـيم فعاليـة البكتيريـا (باسـيليس سـبتاتليس) ومقارنه بالمبيد فلدزيم   ضد نمو الفطـر (فيـوزيري اكسسـبوريم) فـي الطمـاطم. الفطـر الذي تمت تربيته في بيئة البطاطس ديكسـنروز اجـارفي المعمـل والفطـر مهـم فـي انـه يسبب نقص معنوي في هذه الدراسه نم عزل المسبب المرضي مـن النباتـات المصـابة وتـم التعرف عليه من خلال نموه فـي المزرعـه وتـم التعـرف علـي انـه فطـر الفييوزيـوريم اكسسبوريم استخدمت البكتريا كمضاد لنمو الفطريـات مقارنـة مـع المبيـد وكانـت النتـائج بان المبيد تثبيطه عالي ١٠٠%لنمـو الفطـر وتبعتـه البكتريـا باسـيليس سـبيتاليس لاتوجـد فروقات معنوية بين نمو الفطرمع الباسليس سبيتاليس ونمو الفطر في الكنترول

CHAPTER ONE

INTRODUCTION

-Tomato is a tender a warm season perennial cultivated as an annual; it is an annual shrubby member of solanaceae.

In Sudan they are fifteen states cultivating tomato crop, but the main products area are Gezira, Khartoum, and Nile state. Tomato cultivated in both open filed and greenhouses. It is the second popular vegetables after onion in Sudan (Abdol hafeez*, et al*. 2010).

In the arid to tropical region of the Sudan the high summer and the low relative humidity limits the production of tomato to the cooler period of the year. To extend the season of production it is necessary to know the nature of growth, flowing and fruiting of the plant in relation to climatic condition (Abdalla and Verkerk, 1968).

Tomato is major vegetable crop in Sudan' the major varieties of tomato growing in the Sudan are strain B' and peto 86' since they tolerate high temperature. Several diseases that affect tomato , these include, Bacterial canker, Bacterial spots, Early blights, Anthracnose, Verticillum wilt and Powdery mildew Hence the occurrence of Fusarium wilt disease the causal agent is (*Fusarium oxysporum*) has been observed in recent years in Khartoum area with high levels of severity.

The thesis is therefore intended to:

To investigate the efficacy of alternative control measures involving the biological agents *Bacillus subtitles*.

CHAPTER TWO

2.1 Tomato Plant

Tomato (*Solanum lycopersicum L)* is the edible, often red fruit from the plant, commonly known as a tomato plant. The tomato is consumed in diverse ways, including raw, as an ingredient in many dishes, sauces, salads, and drinks. While it is botanically a fruit, it is considered a vegetable, accounts for 14% of world vegetable production over 100 million metric tons/year$ 1.6 billion market (Food and Agriculture Organization FAO, 2010). The total production year 1999 was 707715 tons and the total production of tomato for one greenhouse (350m) in Khartoum reached 5ton per season (Abdol hafeez*, et al*. 2012).

2.1.1Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Solanales

Family: Solanaceae

Subfamliy: Solanoideae

Tribe: Solaneae

S.N: *Solanum lycopersicum (L)(Raabe et al 2014*)Distribution:

The written literature of tomato began in 1500 when Spanish and Portuguese explorers found these plant first in mexico and then along the west coast of south America mainly peru, and then along on the Galapagos island, tomato is a native to peru-equador regain of south America, evolving from the cherry from (Iycopersicon esculantum Var. cerasiform).(Perice,1987).

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In the arid to tropical region of the Sudan the high summer and the low relative humidity limits the production of tomato to the cooler period of the year. To extend the season of production it is necessary to know the nature of growth, flowing and fruiting of the plant in relation to climatic condition (Abdalla and Verkerk, 1968)

2.1.2 Varieties

There are around 7500 tomato varieties grown for various purposes,Heirloom tomatoes are becoming increasingly popular, particularly among home gardeners and organic producers, since they tend to produce more interesting and flavourful crops at the cost of disease resistance and Productivity (Redenbaugh,*et al*1992).

The tomato is now growing worldwide for its edible fruits, with thousands of cultivars having been selected with varying fruit types, and for optimum growth in differing growing conditions. Cultivated tomatoes vary in size about5mmin diameter, through cherry tomatoes, about the Same (1-2cm) size as the wild tomato, up beefsteak tomatoes (10cm)or more in diameter, the most widely grown commercial tomatoes tend to be in the (5-6cm) diameter range.

Most cultivars produce red fruit, potato number of cultivars with yellow, orange, pink, purple, green, black, or White fruit are also available. Multicoloured and striped fruit can also be Quite striking .T0matoes grown for canning and sauces are often elongated, (7-9cm) long and (4-5cm) diameter, they are known as plum

Tomatoes, and have a lower water content. Roma-type tomatoes are important cultivars in the Sacramento Valley (Redenbaugh, *et al*1992).

The tomato varieties for summer season such as: Eloths and Sophie, Areas in Sudan 58400fedans in1999 (Mohamed *et al*.2003).There are other resistance type breeding in Sudan against tomato yellow leaf curl Viruses includes, Sennar (1) Sennar (2), Omdurman, and Alijazeera (96).Variety Abed Allah and Somerset (98) are breeding to resist high Temperature in Sudan(Ahmed,2009).

2.1.3 Impotence and Nutrition value of tomato

Tomato is considered as an importance source of some vitamins and Mineral salt such as; vitamin B, and Riboflavin, which are considered necessary for growing, and safety of skin .The external part of fruit contains high level of vitamin C. This for red tomato, raw(per100g:energy74kg, carbohydrates3.9g, fat 0.2, protein 0.9, vitamin5% and trace metals 3%).their also others constituents such as water94.5and lycopene 2573mg (Naika, *et al.* 2005).which are considered necessary for growing and safety of skin. The external part of fruit is Contains light level of vitamin C (Alaa Edrees, 2014).

2.1.4 The Diseases

Tomato cultivars vary widely in their resistance to disease. Modern Hybrids focus on improving disease resistance over the heirloom plants. One common tomato disease is tobacco mosaic virus, so smoking or use of tobacco products are discouraged around tomato, over whether the virus could possibly survive being burned and converted into smoke. Various formed of mildew and blight is also common tomato afflictions, which is why tomato cultivars are often marked with combination of? Letters is referring to specific disease resistance. The most common letters are Verticillium wilt, F wilt strain Land II, Nematodes, Tobacco mosaic virus, Alternaria solani (Mourvaki, *et al* .2005).Tomato attacks by many diseases and pest in Sudan, the important Disease in Sudan include; Damping off-of seedling, tomato yellow leaf Curl viruses (TYLCV), powdery mildew, Bacterial spot, early and late Blight and Fusarium wilt (Juha, 1996).

2.2 Fusarium wilt of Tomato

Fusarium is a major genus of soil Fungi that is found in many parts of the world most species are harmless saprobes and are relatively abundant members of the soil microbial community. Some Fusarium species are economically significant due to the devastating impact they can have on crops. (Abdol Hafeez, *et al* 2012)

2.2.1 Scientific classifications of Fusarium wilt of Tomato

Kingdom: Fungi

Phylum: Ascomycota

Class: Sordariomycetes

Subclass: Hypocreomycetidae

Order: Hypocreales

Family: Nectriaceae

S.N: *Fusarium oxysporum f.sp. lycopersici*. (Raabe, *et al* 2014)

2.2.2 HOSTS of the pathogen

The fungal pathogen Fusarium oxysporum affects a wide variety of hosts of any age. Tomato, tobacco, legumes, cucurbits, sweet potatoes and banana are a few of the most susceptible plants, but it will also infect other herbaceous plants. (Agrios, et al2005).

2.2.3 Symptoms

Fusarium oxysporum generally produces symptoms such as wilting, chlorotic, and necrosis, premature leaf drop, browning of the vascular system, stunting, and damping off. The most important of these is vascular wilt (Agrios, et al2005).

Fusarium wilt starts out looking like vein clearing on the younger leaves and drooping of the older lower leaves, followed by stunting of the plant, yellowing of the lower leaves, defoliation, marginal necrosis and death of the plant. On older plants, symptoms are more distinct between the blossoming and fruit maturation stages.

The life cycle of *F. oxysporum* commences with a saprophytic phase when the fungus survives in soil as chlamydospores (Beckman & Roberts 1995). Chlamydospores remain dormant and immobile in the remains of decayed plant tissue until stimulated to Germinate by utilising nutrients that are released from extending roots of a variety of plants (Stover 1962 Beckman and Roberts 1995). Following germination, a thallus is produced from which conidia form in 6-8 hours, and chlamydospores in 2-3 days if conditions are favourable. Invasion of the roots is followed by the penetration of the epidermal cells of a host or a non-host (Beckman and Roberts 1995) and the development of a systemic vascular disease in host plants (Stover, 1970). In the advanced stages of the disease, the fungus grows out of the vascular system into adjacent parenchyma cells, producing vast quantities of conidia and chlamydospores. The pathogen survives in infected plant debris in the soil as mycelium and in all its spore forms, but most commonly as chlamydospores in the cooler temperate regions (Agrios 2005).

2.2.4 Distribution

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

2.2.5 Biology

In solid media culture, such as potato dextrose agar (PDA), the different special forms of f. oxysporum can have varying appearances. In general, the aerial mycelium first appears white, and then may change to a variety of colours- ranging from violet to dark purple- according to the strain(or special form) of F. oxysporum. If sporodochia are abundant, the culture may appear cream or orange in colour (Smith *et al*., 1988).

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

2.2.6 Epidemiology

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

2.2.7 Management

F .oxysporum and its many special forms affect a wide variety of hosts; the management of this pathogen is discussed in more detail in the respective summaries. In general, some effective means of controlling *F .oxysporum* include: disinfestations of the soil and planting material with fungicidal chemicals, crop rotation with non-hosts of the fungus, or by using resistant cultivars (Jones etales., 1982; Agrios, 1988; Smith etales., 1988).

2.2.8 Controls

The control of *F .oxysporum* of tomato is important in maintaining plant vigour. Documented methods that are used in the control of the disease cultural, biological, use of resistance and use of natural products however, each method has got its own strengths and imitations.

2.2.8.1 Use of resistance

The most cost-effective and environmentally safe method of control is the use of resistant cultivars whenever they are available. The use of resistant varieties is the best strategy for disease control according to (Pritesh *et al* 2011), identification and utilization of tomato plant varieties resistant to the disease represents a valid alternative to the use of chemicals. However, breeding for resistance can be very difficult when no dominant gene is known. In addition, new races of pathogens overcoming host resistance can develop.

The advantages of this method include saving the cost of chemical for control of the disease and enhancing cultivation of previously infested fields.

Chemical application to soil and resistant cultivars are the main approaches to control the disease (Fravel *et al*. 2003).However, fungicide application is often ineffective as the chemical may not reach the

Fungal prop gules which is widely disinfected the soil (Campbell 1989). In addition, new races of the pathogen have overcome host resistance and

Discovery of new resistant varieties is expensive and difficult when no dominant gene is known (Fravel *et al*. 2003). Biological control, therefore, holds promise as a strategy for disease management

.Bio control agents (BCAs) including fluorescent Pseudomonas, a non-pathogenic Fusarium strain,

Trichoderma harzianum and *T. asperellum* have been reported to provide control of Fusarium wilt (Larkin and Fravel 1998; Cotxarrera et al. 2002; Yigit and Dikilitas 2007).

2.3 Bacillus subtilis

Bacillus subtilis, known also at the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium, found in soil and the gastrointestinal tract of ruminants and humans. A member of the genus *Bacillus*, *B.subtilis* is rod- shaped, and can form a tough, protective endospore, allowing it to tolerate extreme environmental conditions. *B.subtilis* has historically been classified as an obligate aerobe, though evidence exists that it is a facultative anaerobe. *B.subtilis* is considered the best studied Gram-positive bacterium and a model organism to study bacterial chromosome replication and cell differentiation. It is one of the bacterial champions in secreted enzyme production and used on an industrial scale by biotechnology companied. (Nihorimbere V, *et al* 2010).

2.3.1 Scientific classification

Domain: Bacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Genus: *Bacillus*

Species: *B.subtilis* (Ehrenberg 1835) Cohn 1872

2.3.2 Habitat

This species is commonly found in the upper layers of the soil, and evidence exists that *B.subtilis* is a normal gut commensal in humans. A 2009 study compared the density of spores found in soil (about 106 spores per gram) to that found in human feces (about 104 spores per gram). The number of spores found in the human gut was too high to be attributed solely to contamination. (Kobayashi, *et al*.2011).

2.3.3 Characteristics

Like all members of the genus *Bacillus, B.subtilis* is a rod-shaped bacterium that typically forms small clumps, short chains, or single cells. It has a cell wall that is made of a complex molecule called peptidoglycan, which is made of Long chains of glucose linked together by amino acids. Peptidoglycan surrounds the cell membrane and gives the bacterial cell shape and structure.(Ferreira,Matthee,*et al*2014). Morphological and biochemical tests were done to characterize rhizobacteria using standard procedure (16). For fame analysis, strains of B.subtilis DSBA-5, showing antagonistic and plant growth promoting ability,were grown in TSA (trypticase soya agar)medium at 28

2.3.4 Application to Biotechnology

Bacillus organisms, isolated by soil sprinkle technique, are responsible for producing antibiotic activity was seen in *Bacillus subtilis* MH-4. The antibiotic bacitracin was determined to be affective on Gram-positive bacteria only (Jamil 2013).*Bacillus subtilis* bacteria secrete enzymes, such as amylase, lipase, among others. These enzymes are produced commercially and this enzyme production represents about 60% of the commercially produced industrial enzymes (Morikawa 2012).

2.3.5 Pathology

Bacillus subtilis bacteria are non-pathogenic. They can contaminate food; however, they seldom result in food poisoning. They are used on plant as fungicide. They are also used on agricultural seeds, such as vegetable and soybean seeds, as a fungicide. The bacteria, colonized on root systems, compete with disease causing fungal organisms. *Bacillus subtilis* use as a fungicide fortunately does not affect humans. (Tan S, *et al* 2013).

CHAPER THREE

MATRIALS AND METHODS

This study which conducted under laboratory conditions was carried out at Plant pathology laboratory of plant protection department college of Agricultural studies Sudan University of science and technology to evaluate the efficacy of Bacillus *subtitles* and (Fulldazim 50 WP) fungicides against the growth of *Fusarium oxysporum f.s lycopersion* in tomato in culture media Potato dextrose agar (PDA) *in vitro*.

3.1.1 Isolation of *Fusarium oxysporum*

Fusarium oxysporum is the causal agent of tomato wilt.

3.1.2 Isolation from plant materials

Infected tomato fruit showing symptom of the disease were obtained from Research Botry Station in Agues, 2017.The fruits were cut into small Sections (0.5-1.0 cm), washed thoroughly with tap water, surface sterilized with Clorox (Naocl) for 5minutes, rinsed three time in changes of sterilized distilled water and dried on sterilized filter papers. The sterilized fruits section were plated at the rate of five sections/plate onto potato dextrose agar (PDA) medium supplemented with chloramphenicol (0.05 g/L)in 9-cm Petri dishes. The Petri dishes were incubated at 25C. After incubation for 7days, isolated fungus was sub cultured on PDA. When free from contamination; Isolates were maintained on PDA slants and examined visually for their growth patterns and pigmentation on the adverse side of the agar. Further microscopic examinations were carried for mycelia and conidia structure using pure of *Fusarium oxysporum* f.sp .lycopersion was obtained by using hyphae tip technique. Pure culture of the isolated fungi was transferred to PDA slants and kept in refrigerator at 40 c for further use

.Sample of the obtained colonies were sub cultured by transferring small mycelia from the colony margins .Pure cultures were obtained by sub – culturing three time and slides were prepared and examined microscopically to confirm identity (x:40)

3.1.3 Identification of the pathogen

The indention of the fungus was based on visual culture characteristics, mainly the growth patterns and pigmentation, furthermore, microscopic examinations were carried out for mycelia and conidia structure based on method of Booth key (1977).

3.1.4 Growth Rate of the pathogen

The pure cultures of *Fusarium oxysporum* were prepared using 7days old mycelia. The fungus was cultured on PDA then transferred, aseptically, to the canter of Petri dishes containing PDA medium and incubated at 25c. The linear growth of the fungus was assessed in cm after 48h.

3.2 Effects of *Bacillus subtilis* on the growth of *F. oxysporum in vitro*

The experiment was laid out in Petri dishes containing sterilized PDA. Half of the solidified medium was inoculated with the fungus from 7 days old culture as in 1 cm discs. The second half was inoculated with a *Bacillus subtilis*. Thus, both organisms would get equal opportunities for growth. One plate containing the test fungus only was included as control. The individual treatments were replicated three times. The Petri dishes were incubated at 25^oC and fungal growth was estimated daily and percent growth inhibition was calculated as in 3.1.4.

CHAPTER FOUR

RESULTS

The results (Figure 1) two days after inculcation indicated that the growth of the fungal decreased by the growth of bacteria compared to the control (1-4.6cm). Four days after inculcated the results indicated that the growth of the fungal increased but the growth of bacteria decreased compared to the control (3.9-6.3) the resulted as in the plate1 and two.

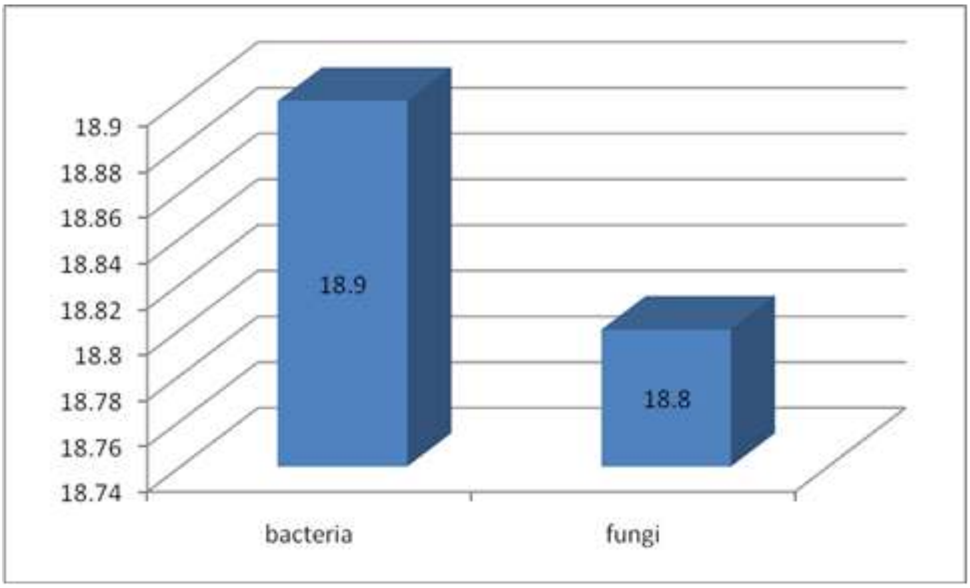

Fig* 1 Effects of *Bacillus subtilis* on leaner growth of *F. Oxysporum in vitro

Plat1:Bacterial and Fungal

Plat2: Effect of Fungicide

CHAPTER FIVE

DISCUSSION

Wilt disease is an economically important disease on tomato in Sudan. This work is a participation aiming at solving the problem of wilt disease in Sudan. Biological control may be defined as the reduction in inoculum density or disease producing activity of pathogen in its active or dormant state, by one or more organisms naturally or through manipulation of environment and host of the antagonists (Baker and Cook, 2010).

Bio control agents are widely regarded by the general public as ‘natural’ and therefore non-threatening products, although risk assessments must clearly be carried out on their effects on non-target organisms. Moreover, Knowledge concerning the behaviour of such antagonists is essential their effective use.

The results indicated that *Fusarium oxysporum* (18.8) and Bacillus subtilis (18.9).The pathogens grown fully that means the *Bacillus subtilis* don't reduced the fungus there are no effect.

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