

CHAPTER ONE

INTRODUCTION

Potato plant (*Solanum tuberosum* L.) is a member of the family Solanaceae that includes eggplant, tobacco and tomato. The crop which was originally believed to be domesticated independently in multiple locations is an important crop worldwide and ranks fourth in production among food crops after maize (*Zea mays* L.), rice (*Oryza sativa* L.), and wheat (*Triticum aestivum* L.) (FAOSTAT data, 2006). The importance of potatoes is increasing due to the rising world population. The potatoes can grow well in diverse conditions, with high nutritional value with an annual production of 3.6 x10⁸ tones (Hamilton, 2005 and Anonymous,2012).

In Sudan, although potato cultivation depends mainly on exotic advanced cultivars but an old introduced material is still produced in Jebel Marra in the far west and it is locally known as Zalingei potato. The crop is cultivated in small area around large cities along the Nile and on seasonally flooded plains (FAO, 1999). However, the area around Khartoum accounts for over 70 percent of the country's potato production (Geneif, 1986).

The losses caused by diseases and insects constitute the major constraints that facing the production of potato worldwide and among these, the most important wide spread and important are pathogenic fungi, affecting tubers and vegetative parts. One of the main fungal pathogens that attack potato is Fusarium dry rot which is a worldwide economic problem. There are many species of Fusarium reported to cause dry rot of potato Worldwide (Nielsen, 1981) of which *Fusarium solani* has been reported

as the most pathogenic *Fusarium* species causing potato dry rot (Sharifi *et al.*, 2009; Soheili-Moghadam and Hosseinzadeh, 2013).

The disease affects tubers in storage and seed potato pieces after planting. Hanson *et al.*, (1996) reported that *Fusarium* dry rot of feed tubers can cause crop losses up to 25%, while more than 60% of tubers can be infected in storage. Indiscriminate use of chemical pesticides to control various pests and pathogenic microorganisms of crops plants is causing health hazard both in terrestrial and aquatic lives through their residual toxicity (Viana *et al.*, 1996; Nikan and Morowati, 2013). Much attention is being focused on the alternative methods of pest control (Ali, 1996).

Natural plant extracts have been recommended as suitable alternative choices to synthetic chemicals (Suhr & Nielsen, 2003; Babu *et al.*, 2008; Ownagh *et al.*, 2010; Bahraminejad *et al.*, 2010; Mangang and Chetry, 2012; Jafarpour *et al.*, 2013) to control diseases and pests of crops. In Sudan the bioactivity of many cultivated and wild Plants are demonstrated by many researchers (El-kamali 2001; Al-Doghairi *et al.*, 2004; and Sidahmed *et al.*, 2009).

Based on the foregoing and considering the adverse and alarming effects of synthetic pesticides on environment and natural habitats, this study was undertaken to find out an alternative and nontoxic biological control agents to control the dry rot of *Fusarium* in potatoes. It is aimed at investigating the antifungal activity of some higher plant extracts and

fungicide (Revus Top) against Fusarium dry rot of potato under laboratory conditions with following objectives:-

- To explore the antifungal potentials of some higher plants crude extract against Fusarium dry rot of potato
- To evaluate the efficacy of systemic fungicide on fungal growth

CHAPTER TWO

LITERATURE REVIEW

2.1. Potato (*Solanum tuberosum* L.)

The potato plant which belongs to the family Solanaceae includes, among 2000 other species, tomato (*Lycopersicon esculentum* L.), sweet pepper (*Capsicum annuum* L.), eggplant (*S. Melongena* var. *esculentum* L.), tobacco (*Nicotiana tabacum* L.), and petunia (*Petunia hybrid* L.) (Fernald, 1970).

2.1.1. Scientific classification

Kingdom: Plantae (unranked):

Order: Solanales

Family: Solanaceae

Genus: Solanum

Species: *tuberosum*

(Binomial name: *Solanum tuberosum* L.)

The genus Solanum is a polymorphous and largely tropical and subtropical genus containing more than 1000 species. The origin agreed to be the high elevation of South America and the area of first domestication was reasoned to be the area where wild diploids are still found and where the greatest diversity of cultivated forms can still be found, and is identified as the high plateau of Bolivia and Peru, in the general region of Lake Titicaca (Hoopes and Plaisted, 1987).

Potato is one of the major vegetable crops grown worldwide following

wheat, maize, and rice, with a production estimates of 368 million tons (FAOSTAT, 2015). It is the staple food of many cultures and civilizations past and present. The term Potato is used to refer both to the plant, and the vegetable itself (Howard *et al.*, 1970).

In Sudan, the potato is grown mainly as winter crop and the main area of production are along the Nile bank in both Khartoum and Northern Estates. Although potato cultivation in Sudan depends mainly on exotic advanced cultivars but an old introduced material is still produced in Jebel Marra in the far west and it is locally known as Zalingei potato (Abdelgadir, 2005).

Potatoes in Sudan are an important cash crop for small-scale growers, and have the potential to increase incomes in periurban areas, improve living standards and create employment opportunities. Potato production is steadily increasing in Khartoum; the acreage devoted to this crop has more than tripled in the last ten years (Ahmed, 1985).

The total acreage under potato cultivation in the Khartoum region amounts to about 6,500 hectares, with yields of 17 to 25 ton/ha. However, crops. Seed potatoes have to be imported and account for more than half of the total production cost of potatoes (Elsir, 2005). This is a major constraint to further expansion of potato production (Elrasheed and Ballal, 2009). The estimated total potatoes production in Sudan is about 616,000 tons in a cultivated area of about 88,000 feddans (Hind and Mohamed, 2010).

One of the major constraints facing the quantity, quality and availability of healthy crop worldwide are the losses and contamination caused by post harvest diseases. The major groups of postharvest diseases are those which arise from infections initiated during and after harvest.

The threat to potatoes from fungal infections has now reached a level that outstrips that posed by bacterial and viral diseases (Berger, 1977). One of the main fungal pathogens that attack potatoes is *Fusarium* spp which are a worldwide economic problem (Nielson, 1981).

Economic importance;

- The potato is a starchy, tuberous crop from the perennial *Solanum tuberosum* of the Solanaceae family (also known as the nightshades).
- The word potato may refer to the plant itself as well as the edible tuber.
- In the region of the Andes ,there are some other closely related cultivated potato species.
- Potatoes are the worlds fourth largest food crop, following rice, wheat, and maize.
- Long-term storage of potatoes requires specialized care in cold warehouses and such warehouses are among the oldest and largest storage facilities for perishable goods in the world.
- Once established in Europe, the potato soon became an important food staple and field crop.
- The annual diet on of an average global citizen in the first decade of the twenty-first century included about 33 kg (or 73 Ib) of potato.
- However, the local importance of potato is extremely variable and rapidly changing.

- It remains an essential crop in Europe, where per capita production is still the highest in the world, but the most rapid expansion over the past few decades has occurred in southern and eastern Asia.
- China is now the world's largest potato-producing country, and nearly a third of the world's potatoes are harvested in China and India (Thompson and Morgan, 1855).

2.2. Fusarium dry rot

Fusarium dry rot of potato is a devastating post-harvest disease affecting both seed potatoes and potatoes for human consumption. In fact, Fusarium dry rot of potatoes is a worldwide economic problem. There are many species of *Fusarium* reported to cause dry rot of potato worldwide (Nielson, 1981). The disease may cause greater losses of potatoes than any other post-harvest disease. Crop losses attributed to dry rot have been estimated to an average of 6 to 25% (Powelson *et al.*, 1993).

Fusarium species which cause dry rots are also important to the consumer because some *Fusarium* which cause dry rots also produce mycotoxins, one of such toxins is trichothecene which is an inhibitor of eukaryotic protein synthesis and can pose serious health problems to man and animals (Beremaid *et al.*, 1991).

This fungus which prefers warmer climates causes a variety of colored rots in potatoes (Rowe *et al.*, 2013). There are many species of *Fusarium* reported to cause dry rot of potato worldwide of which *Fusarium solani* has been reported as the most pathogenic *Fusarium* species causing potato dry rot (Sharifi *et al.*, 2009).

2.2.1.Classification

Kingdom:	Fungi
Phylum:	Ascomycota
Class:	Sordariomycetes
Subclass:	Hypocreomycetidae
Order:	Hypocreales
Family:	Nectriaceae
Genus:	<i>Fusarium</i>
Species:	<i>solani</i>

2.2.2.Host range and distribution:

The predominant hosts for *Fusarium solani* are potato, pea, bean, and members of the cucurbit family such as melon, cucumber, and pumpkin. Some strains may cause infections in humans. Fusarium damping-off, corn rot, fruit rot, root rot, and surface rot are caused by *F. solani f.sp.eumartii* (Aoki *et al.*,2003).

Mart, Saccand found in most states in the United States. the fungus has a worldwide distribution, but its frequency as an important plant pathogen is well known and hence remains the most common disease-causing fungus in its genus (Aoki, *et al.*, 2003).

2.2.3.Phylogeny;

The phylogeny of isolates from potato and tomato was determined based on sequences of two DNA fregments: rDNA internal transcribed spacer regions and partial sequences of elongation factor 1- a. All isolates of *F.solani.f.sp.eumartii*, from tomato and potato formed a single monophyletic clade distinct from other formae speciales and mating population of *F.solani.f.sp.eumartii*,The results of this study demonstrates

that Eumartii wilt and tomato foot rot in California both are caused by *F.solani.f.sp.eumartii*, (Romberg and Davis, 2007).

2.2.4. Description:

Fusarium solani is a filamentous fungus in the genus *Fusarium*, and the anamorph of *Haematonectria aematococca*. *Fusarium solani* (Mart.) Sacc.(2008). is a name that has been applied broadly to what is now known as the *F. solani* species complex (FSSC; O'Donnell 2000). Members of the FSSC, which includes several additional named species and currently corresponds to approximately 50 phylogenetic species (Zhang *et al.*, 2006; O'Donnell *et al.*, 2008), are ubiquitous in soil, plant debris and in other plant and animal substrata and can be serious plant and human pathogens (Booth, 1971). The FSSC contains both heterothallic and homothallic strains and species, as well as strains that have no known sexual stage. The fungus produces three types of asexual spores, micro conidia, macro conidia and chlamydia spores. The macro conidia are straight to slightly curved, slender thin walled usually with three or four septa, of a foot shaped cell. They are generally produced on conidiophores by division. They are important in secondary infection. The micro conidia are ellipsoidal and either have no septum or single one. They are formed from phialides in false heads by secondary infection (Agrios, 2005).

The chlamydispores are globes and have thick walls. It is formed from hyphae or alternatively by the modification of micro cells. Conidia considered as endurance organs in soil where they act as inoculum in primary infection.

The telemorph or sexual reproductive stage of *F. oxysporum* is unknown.(Booth, (1977) stated that the chromosome number of the

fungus is (12) and the perithecial state is Gibberella but not confirmed (Agrios, 2005).

2.2.5.Causal organism:

Several Fusarium spp., including *F.sambucinum*, *F.solani* var *.coeruleum*, *F.avenaceum*, can cause dry rot. These fungi survive on refuse and live in soil. Infections can originate from infected seed tubers. Tuber rot usually does occur unless the tuber is injured during harvest. Wound provide a way for the fungus associated with soil to enter the tuber. Dry rot is one of the common storage diseases in Idaho. Fusarium dry rot lead to secondary infections by soft rot bacteria (Ocamp *et al.*, 2006).

2.2.6. Symptoms :

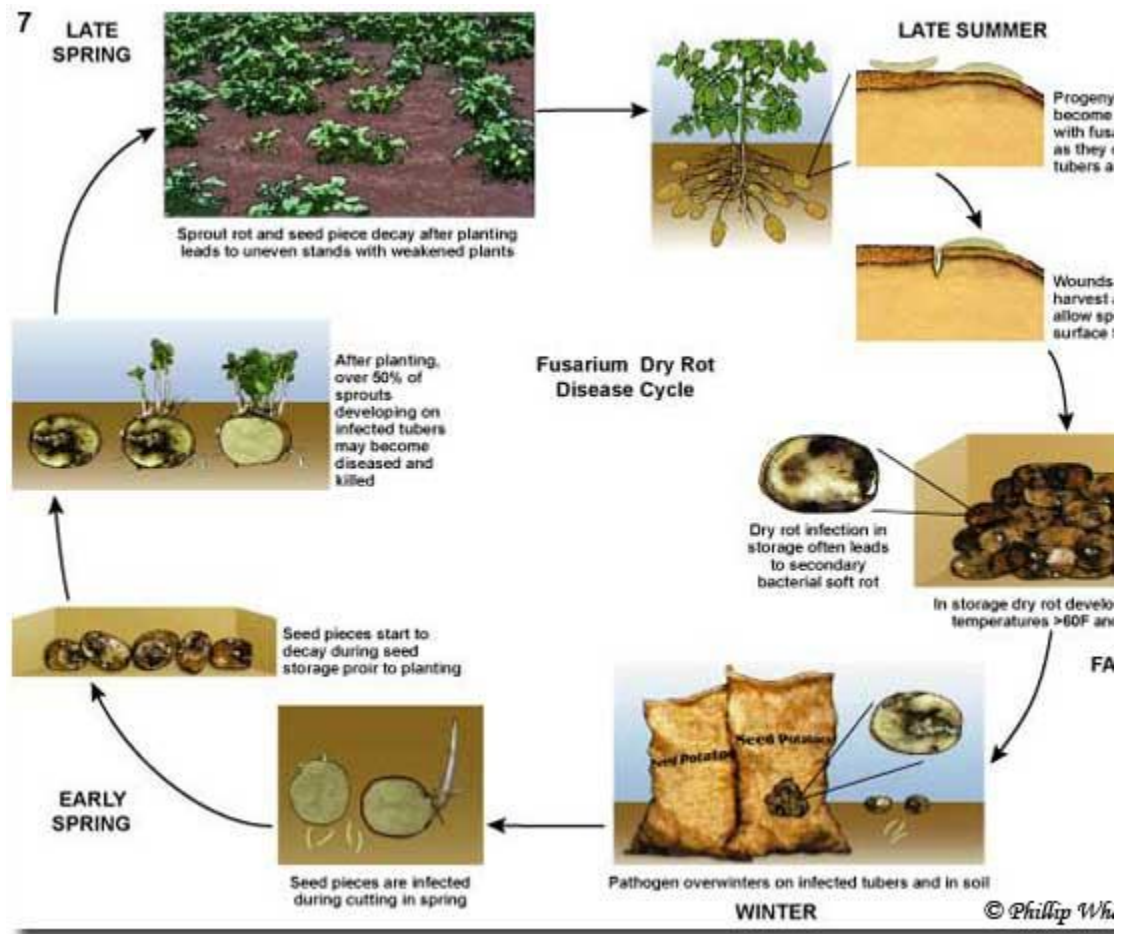
The fungus can be soil borne, airborne or carried in plant residue and can be recovered from any part of the plant from the deepest root to the highest flower (Summerral *et al.*, 2003).

Fusarium dry rot of potato is a devastating post-harvest losses disease affecting both seed potatoes and potatoes for human consumption (Loria and Rosemary, 1993). Dry rot causes the skin of the tuber to wrinkle. The rotted areas of the potato may be brown, grey, or black and the rot creates depressions in the surface of the tuber. Seed pieces may rot completely before they have the chance to be planted. Signs of a pathogenic Fusarium species can be seen on an infected potato, and include white or pink mycelia (masses of vegetative fungal tissue) and very colorful spores that can be blue, black, purple, grey, white, yellow, or pink (Loria and Rosemary, 1993).

2.2.7.Disease Cycle:

Fusarium dry rot is caused by several fungal species in genus *Fusarium*. *F. sambucinum* (teleomorph *Giberella pulicaris*) is the most common causing dry rot of stored tubers in North America, but other *Fusarium* species are also known to cause dry rot, particularly *F. solani* var. *coeruleum*, and *F. avenaceum*. (Philip and William, 2008).

Fusarium spp. are common in most soils where potatoes are grown and can survive as resistant spores free in the soil for very long periods of time. There are two main opportunities in the potato crop cycle for *Fusarium* spp. to infect potato tubers, in the Spring and in the Fall. *F. sambucinum* and *F. solani*, are commonly found on seed tubers in the spring. Potato seed tubers are maintained at 37°F in storage which is approximately the temperature at which *F. sambucinum* is dormant and consequently there is minimal development of dry rot in storage. However, some level of *Fusarium* dry rot is almost always present in commercially available seed. During the pre-planting phase of potato production seed tubers are warmed to about 54°F then cut into seed-pieces prior to planting. Tubers infected with *F.sambucinum* are particularly susceptible to the development of seed piece decay during this phase and in cases of severe disease, seed pieces may rot completely before planting. Alternatively after planting, over 50% of sprouts developing on infected tubers may become diseased and killed outright before emergence. Damage at this stage results in delayed or non-emergence and is usually expressed as poor and uneven stands with weakened plants. Reduction in crop vigor then results from expenditure of seed energy used to produce secondary or tertiary sprouts to compensate for damage to primary sprouts. (Philip and William, 2008).



Progeny tubers may become contaminated with *Fusarium* spores as they develop in the late summer and early fall. However, they are not usually infected until harvest because the pathogen cannot cause infection unless the the potato skin is ruptured, which rarely occurs during the growing season. Wounds caused during harvest and handling provide dormant spores on the tuber surface with multiple points of entry into the tuber. Once the pathogen has penetrated the tuber skin it begins to grow in the tuber tissue, causing dry rot lesions at the point of entry. In storage, dry rot develops most rapidly at high relative humidity and temperatures of 60 to 70°F. Lower humidity and temperatures retard infection and disease development. However, dry rot may continue to develop at the lowest temperatures safe for storage of potatoes. Young tubers appear to have some resistance to dry rot which slows disease. Dry rot progresses

noticeably faster during the last half of the storage season.(Philip and William, 2008).

2.2.8. Environment (Ecology):

Fusarium solani produces asexual spores (microconidia and macroconidia). Its sexual state is *Nectria haematococca* (Ascomycete), and overwinters as mycelium or spores in infected or dead tissues or seed. It can be spread by air, equipment, and water. (Vincent and Jean, 1971).

Warmer climates are preferred. (Warton *et al.*, 2013). However; different species of *Fusarium* may be more prevalent in different areas. (Rowe *et al.*, 2013). The fungus can persist in the soil for several years. The spores and mycelium are carried into the soil tools. They may also be splashed by rain or carried by floods. The chlamydospore is the survival structure in the absence of a host plant. (Vincent and Jean, 1971).

2.2.9. Importance

Dry rot is not just a cosmetic problem like many other pathogens. It destroys tubers and leaves them completely inedible or unusable as seed in the future. Long-term storage losses have been reported to be as high as 60% while annual dry rot losses can range from 6 to 25% (Gachango and Hanson, (2012). In Michigan, over 50% of seed lots have reported having variable levels of dry rot. (Gachango and Hanson, (2012). *Fusarium* spp., are among the most important plant pathogens in the world and are highly variable because of their genetic makeup and changes in environment in which they grow causing morphological changes (Nelson, 1983).

2.3. Management:

There are many ways to manage dry rot. This includes application of fungicides, cultural practices, sanitation, biological control and botanical

pesticides. However, most techniques for managing dry rot are aimed at preventing injury to the tubers, either seed or the harvested crop. Preventing bruises will greatly aid in avoiding infection (Warton *et al.*, 2007).

2.3.1.Cultural practices:

Cultural practices can also limit the spread of dry rot. Plant high quality seed free from *Fusarium* dry rot pathogens into soils without a history of *Fusarium* dry rot. Varieties vary in their reaction to dry rot, and highly susceptible varieties should be avoided. Harvest tubers at least 14 days after vine kill to promote good skin set and reduce skinning injury that can increase storage dry rot. Avoid harvesting cold tubers that are more susceptible to injury. Provide conditions that promote rapid wound healing early in storage, including high humidity, good aeration, and temperatures of 55 to 64°F for 14 to 21 days. Since *Fusarium* dry rot increases with length in storage, short-term storage is advisable for fields where severe infection is expected (Howard *et al.*, 2005).

2.3.2.Soil solarization ;

Soil disinfestations by soil solarization method at warm season was carried out for the relative control of *Fusarium* pathogens, the main crops cultivated areas, in northwest Iran. In soil solarization method, infested soil was thoroughly plowed to destroy all large clods and remove any existing materials. The soil was irrigated deeply since it was a very important step in the process for increasing transmission of heat through the soil. Then, the moistened soil was covered with transparent polyethylene sheet to raise soil temperatures high enough; more than 10°C above air temperature (Kumar *et al.*, 2002). The plastic edges were buried in the trench to ensure that the plastic is held in place to stabilize the heat. Process was facilitated to raise solar energy and to raise temperature of

moistened soil which can result in the control of soil borne pathogen. The plastics were leaved in treated soil for six weeks during the hottest part of the summer. The method came out to be a successful practice to control soil borne fungi, as well as *Fusarium* species (Pinkas *et al.*, 1984).

2.3.3. Botanical controls;

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

The use of plant extracts for controlling *Fusarium* wilt, cultural practices and the use of other methods are the most common strategies. However, they are either not available or effective. The uses of natural products for the control of fungal diseases in plant are considered as an interesting alternative to synthetic fungicides due to their less negative impacts on the environment. Plant extracts or plant essential oils have been tested against *F.oxysporum* species for inhibitor effect and control efficacy under greenhouse condition (Bowers, and Locke, 2000). If natural plant products can reduce populations of soil borne pathogens and control diseases development, than these plant extracts have potential as environmentally safe alternatives and as component in integrated pest management programs.

2.3.4. Biological control :

Biological control of dry rot is an intriguing concept, but currently nothing is available commercially. Researchers at Michigan State University are investigating the efficacy of *Bacillus subtilis* and *Bacillus pumilis* and *Trichoderma harzianum* in controlling Fusarium dry rot. (Warton and Phillip, 2013). Scientists in Tunisia have found that several bacterial species of the genus Bacillus, commonly found in the salty soils of Tunisia, can reduce the amount of rot seen due to *Fusarium sambucinum*. *Bacillus thuringiensis*, can help control dry rot when applied to older cultures.(Sadfi, 2007).

2.3.5. Chemical control:

Effective chemical control of dry rot can be achieved with chemicals like Tops MZ, Maxim MZ, and Moncoat MZ. These chemicals protect not only against dry rot, but also against other potato diseases like rhizoctonia, silver scurf, and black dot. These chemical treatments can delay emergence of the young plants, but this doesn't mean these chemicals shouldn't be used. Many fungicides, including thiabendazole, work best when they are applied to tubers before they are cut into seed pieces.(Schwartz, *et al*,2005).

CHAPTER THREE

MATERIALS AND METHODS

This study was conducted under laboratory conditions at Plant Pathology Department, College of Agricultural Studies “Shambat”, Sudan University of Science and Technology within the period from January to February 2015. It was intended to investigate the antifungal activity of crude aqueous extracts of Ginger root and Garlic fruit and efficacy of fungicide (Revus top R 56.2) EC, against *Fusarium solani.f.sp.eumartii*.

3.1. Collections of plant samples

Random samples were collected from infected potato tuber showing typical symptoms of dry rot (Loria, 1993) from different farms at Wad Ramli area. Samples were kept in paper sac brought to laboratory for isolation and identification of *Fusarium* dry rot of potato and for further studies.

3.2.1. Isolation of *F. solani* from plant material

Infected potato tubers showing symptom of dry rot disease were cut into small sections (0.5, 1.0 cm), washed thoroughly with tap water, surface sterilized with Clorox (NaOCl) (1% concentration) for 1 minute, rinsed three times in sterilized distilled water and dried on sterilized filter papers. The sterilized sections were then plated at the rate of 6 pieces per plate on to potato dextrose agar medium (PDA).

The inoculated Petri dishes were incubated at 25⁰C for 7days. After incubation, growing fungi were sub cultured on PDA medium for further purification of the fungus. Furthermore, Compound microscopic examinations were carried out for Mycelia and Conidia structure based on the method of Booth, (1977) to confirm that the fungus is *Fusarium solani* Identification of the fungus was supplemented by already prepared slides of *F. solani* at the pathology laboratory. Standard books and

research papers were also consulted during the examination of this fungus (Aneja, 2004). The purified isolates were maintained on PDA medium for further studies.

3.3. Source of plant material.

Garlic fruits and ginger roots were obtained from local market and then brought to the laboratory where they were ready to be used without shading or drying. After that, the plant samples were crushed separately to obtain fine powder from ginger and concentrated suspension from garlic for extraction.

3.3.1. Preparation of crude aqueous extract of plants.

Aqueous extracts of each of the plant materials were prepared as recommended by Okigbo, (2006). The obtained fine powder form of Ginger roots and grinded Garlic bulb plant was weighed (25, 50 and 100 gm) and placed in 100 ml conical flask each and completed to 100 ml sterilized distilled water to obtain the three concentrations and it was placed in a shaker for 4 hrs. The extracts were filtered overnight to obtain 25 % 50% and 100% concentrations.

3.3.2. Preparation of fungicide concentrations.

One ml of the Revus top R (56.2 ia) EC fungicide was dissolved in 100 ml of sterilized distilled water of which 25, 50 and 100 ppm was prepared.

3.4. Test procedure

Inhibition zone technique was used in this study (Rao and Srivastava, 1994) to evaluate the effect of each concentration on mycelial linear growth of the fungus. Initially, fresh fungal growth was prepared from previously maintained culture of *F. solani*. Prepared PDA media was amended with the required concentration from ginger ,garlic and

fungicide before being solidified in a conical flask of 250 ml, agitated and poured into sterilized glass Petri dishes. Three plates, containing 25 ml of PDA, were assigned for each concentration and left to solidify. The other three plates with PDA medium alone were served as control.

One mycelial disc of the fungus was placed in the centre of PDA plates where opposite poles were marked at the back of the plate and incubated at 25°C in incubator and radial growth of pathogen was measured at 24 h intervals from five days.

The Petri dishes of each concentration were arranged in a complete block design in incubator and incubated at 25 C⁰ for 6 days. The growth of the fungus was measured and calculated successively after 3, 4, 5 and 6 days after inoculation. The effect of each extract concentration on linear fungal growth was calculated as percentage of reduction in diameter of fungal growth (R) where: -

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

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

3.5. Experimental design.

The experiment was arranged in a Complete Randomized block Design.

3.6. Statistical analyses.

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

CHAPTER FOUR

RESULTS

This study which conducted under laboratory conditions of Plant Pathology, College of Agricultural Studies, Sudan University of Science and Technology during the period of January to February 2015 to investigate the inhibitory effect of Garlic and Ginger aqueous extracts and Fungicide, Revus top(56.2 ia) EC efficacy against the fungus *Fusarium solani.f.sp.eumartii*.

4.1.Isolation and Identification from the infected sample of potato plant.

The Isolation and Identification of *Fusarium solani.f.sp.eumartii*, were performed according to the shape of spores and conidia.

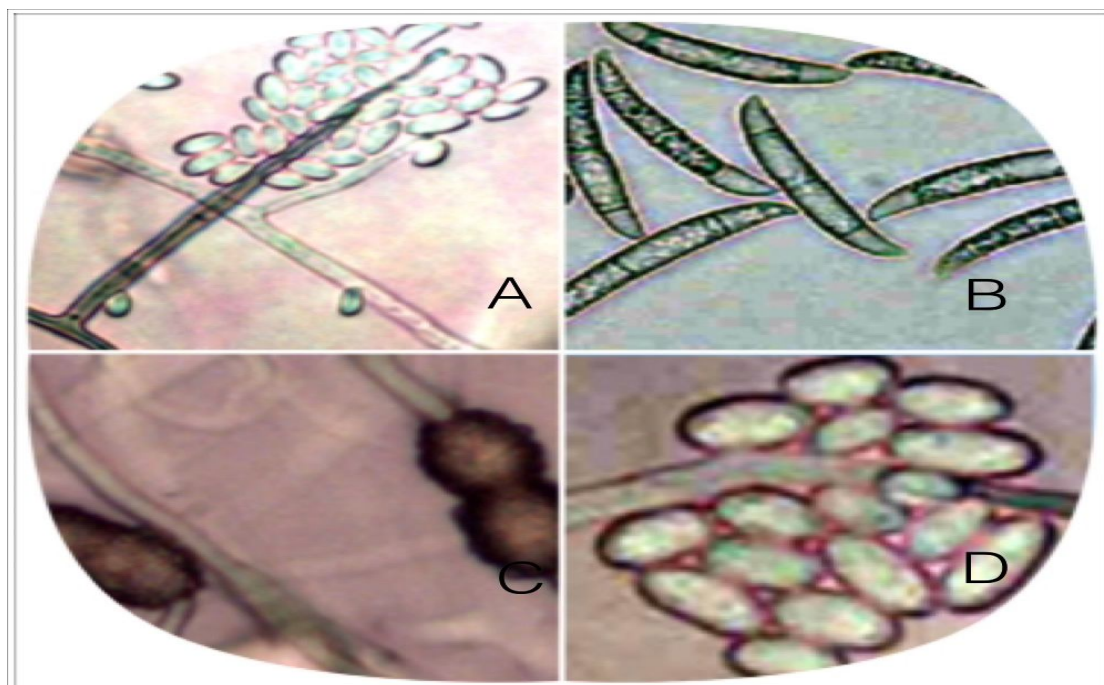


Figure 3. Morphological characters of *F. solani* (Morphotype II). A = Conidiophores, B = Macroconidia, C = Chlamydospores, D = Microconidia (scale bar = 25 μ m).

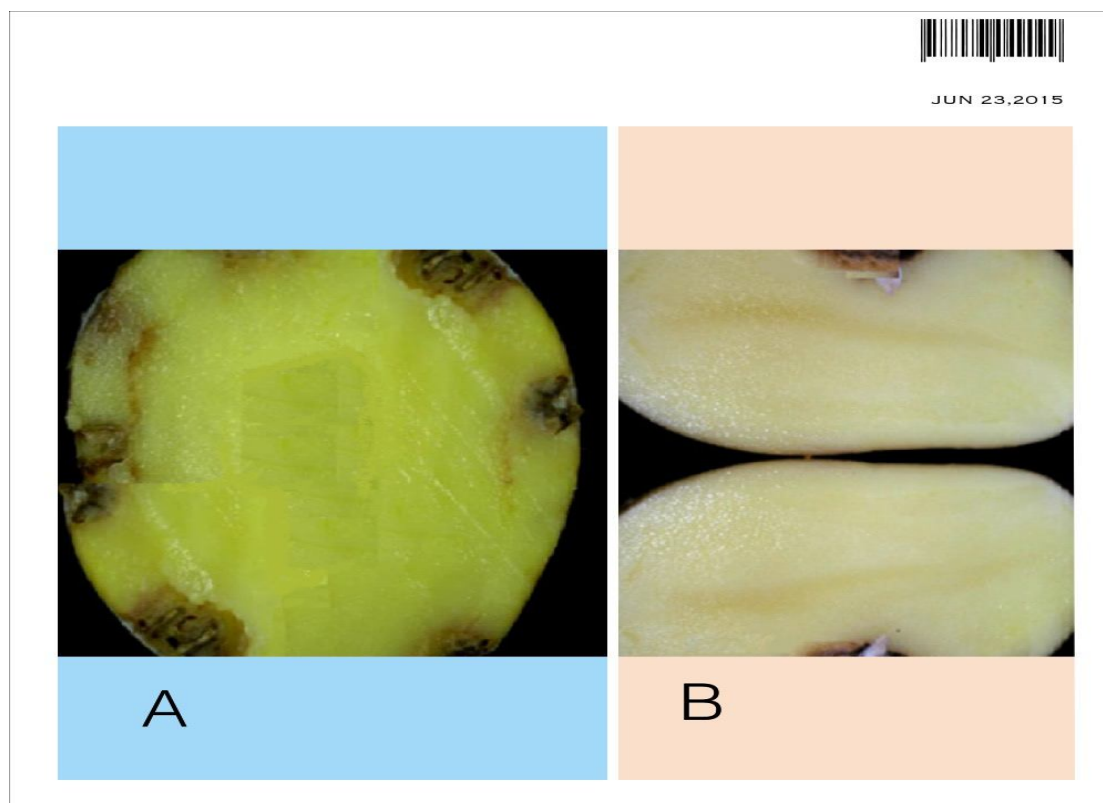


Figure 4. Diseases caused by pathogenic *Fusarium* spp. on potato. A = Dry rot on potato by *F. oxysporum*, B = Dry rot on potato by *F. solani* species complex.

Effect of aqueous extracts of Garlic, Ginger and fungicide(Revus top) on the linear growth of *Fusarium solani.f.sp.eumartii* invitro.

The results (Table 1 and Figure 1) showed that the aqueous extracts of all plants tested and fungicide Revus top R(56.2 ia) EC had effects on the fungal growth after three days from inoculation. Furthermore, the percentages fungal growth inhibition was significantly high compared to the control.

The effect of Garlic fruit (25%, 50%, 100%) gives the Reduction of the growth as 59%, 73%, 95% Respectively, while the effective of Ginger root 25%, 50%, 100% had give the reduction of the growth as 49.3%, 66.3%, 100%.

The Ginger was more effective of the growth on *Fusarium solani f. sp.eumartii*, than Garlic invitro in the first day .but in the second day the Garlic become more effective than Ginger invitro.

Moreover, the highest concentration of the plant aqueous extracts (100%) and Revus top (50 and 100%) gave significantly higher inhibition zones percent (95%, 100%, 94.9 and 100%) respectively compared to the untreated control. Among the plant extracts tested Ginger was invariably the most effective in suppressing the fungus growth than its equivalent Garlic (Table, 1). Generally, the results showed that the antifungal activity increase with increase of extract concentration.

Table, 1: Effect of aqueous extracts of Garlic fruit ,Ginger root and Fungicide Revus top on the linear growth of *Fusarium solani f.sp.eumartii* invitro.(After 3 days inoculation).

Treatments		Inhibition zone (%)			
		R1	R2	R3	Mean
Garlic	25.0%	55.0 (7.0)	63.0 (8.7)	60.0 (7.8)	59.0 (7.7) c
	50.0%	74.0 (7.9)	68.0 (8.0)	78.0 (8.9)	73.0 (8.5) c
	100 %	91.0 (9.6)	100(10.0)	95.0 (9.8)	95.0 (9.7) a
Ginger	25.0%	50.0 (7.0)	48.0 (6.9)	50.0 (7.0)	49.3 (7.0) c
	50.0%	65.0 (8.0)	69.0 (8.3)	65.0 (8.0)	66.3 (8.0) c
	100 %	100(10.0)	100(10.0)	100(10.0)	100(10.0) a
Rivus top	25.0%	89.0 (9.0)	90.0 (9.6)	90.0 (9.6)	89.6 (9.4) b
	50.0%	95.8 (9.8)	94.0 (9.7)	95.6 (9.9)	94.9 (9.7) a
	100 %	100(10.0)	100(10.0)	100(10.0)	100(10.0) a
Control		0.00 (0.7)	0.00 (0.7)	0.00 (0.7)	0.00 (0.7) c
C.V			9.77 (0.0)		
SE			0.620		
LSD			2.064		



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

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

Effect of aqueous extracts of Garlic fruit ,Ginger root and Fungicide (Revus top) on the linear growth of *Fusarium solani f.sp.eumartii* invitro.

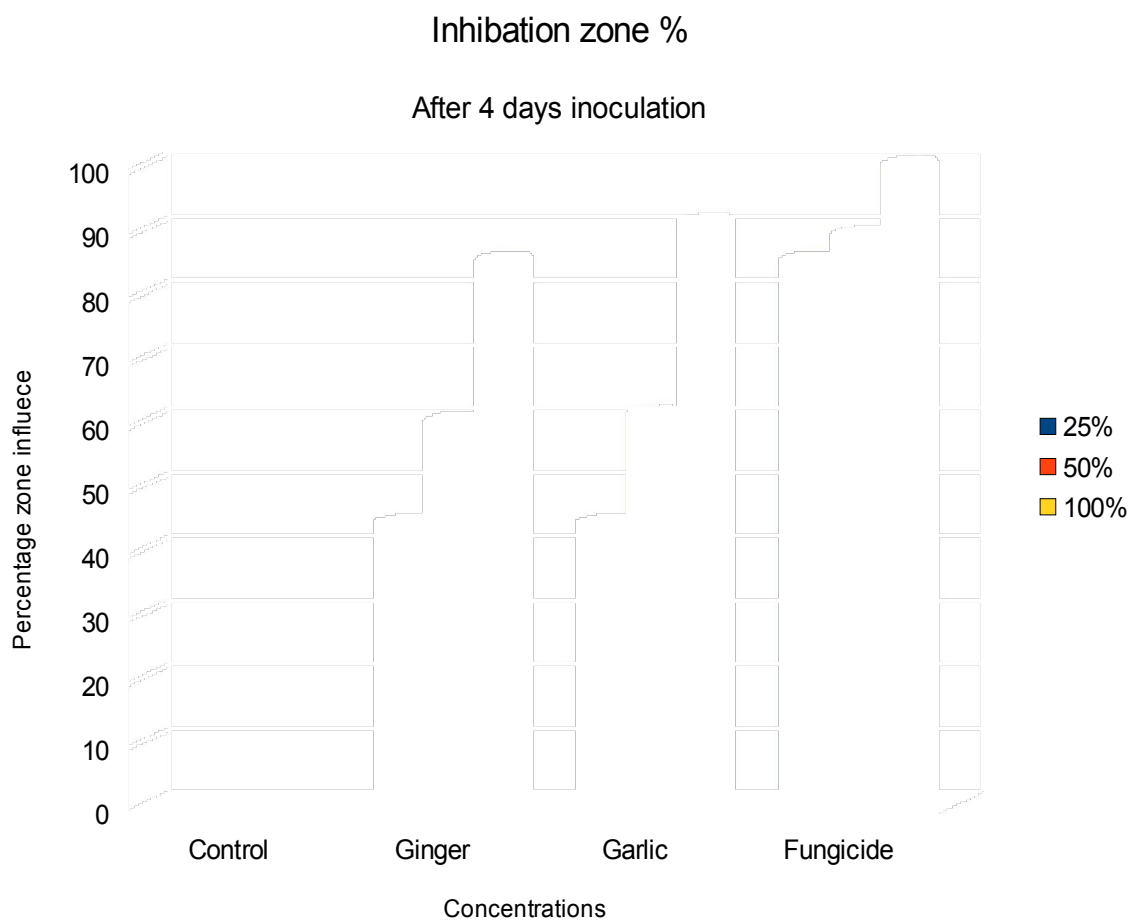
In after three days of inoculation, all plant extracts concentrations as well as that of the fungicide were invariably effective in exhibiting inhibitory the fungal growth.

However, the highest concentration of the plant extracts Garlic fruit ,Ginger root (100%) and Revus top (100%) gave the highest inhibition zones percent (91%, 85.6%, and, 100%) respectively. This inhibitory effect from all concentrations tested was significantly different from control (Table, 2 and Fig. 2). Furthermore, the fungicide irrespective of concentration, (25%, 50% and 100%) induced significant reduction of fungal growth (85.4%, 89.6% and 100%) respectively compared to control.

Furthermore, the Garlic plant extract at all concentrations tested became the most suppressive than Ginger root plant extract, but the fungicide has the highest suppression.

Table, 2: Effect of aqueous extracts of Garlic fruit ,Ginger root and fungicide (Revus top) on the linear growth of *Fusarium solani f.sp.eumartii invitro*.(four days after inoculation).

Treatments Concs. (%)		Inhibition zone (%)			
		R1	R2	R3	Mean
Garlic	25.0%	41.7 (6.0)	35.7 (6.0)	56.0 (7.6)	44.4 (6.0) c
	50.0%	61.7 (7.3)	56.6 (7.8)	72.0 (7.8)	64.4 (8.0) c
	100 %	90.0 (9.5)	94.0 (9.7)	90.0 (9.0)	91.0 (9.5) a
Ginger	25.0%	45.0 (6.7)	40.9 (6.4)	47.6 (6.9)	44.5 (6.7) c
	50.0%	61.7 (7.9)	65.7 (8.0)	61,7 (7.9)	60.0 (7.7) c
	100 %	90.7 (9.5)	80.6 (9.0)	87.0 (9.3)	85.6 (9.2) b
Rivus top	25.0%	86.7 (9.0)	80.6 (9.0)	89.6 (9.5)	85.4 (9.0) b
	50.0%	86.7 (9.0)	82.0 (9.0)	98.9 (9.9)	89.2 (9.4) b
	100 %	100(10.0)	100(10.0)	100(10.0)	100(10.0) a
Control		0.00 (0.7)	0.00 (0.7)	0.00 (0.7)	0.00 (0.7) d
C.V			11.60(0.0)		
SE			0.760(0.0)		
LSD			0.050(0.0)		



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

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

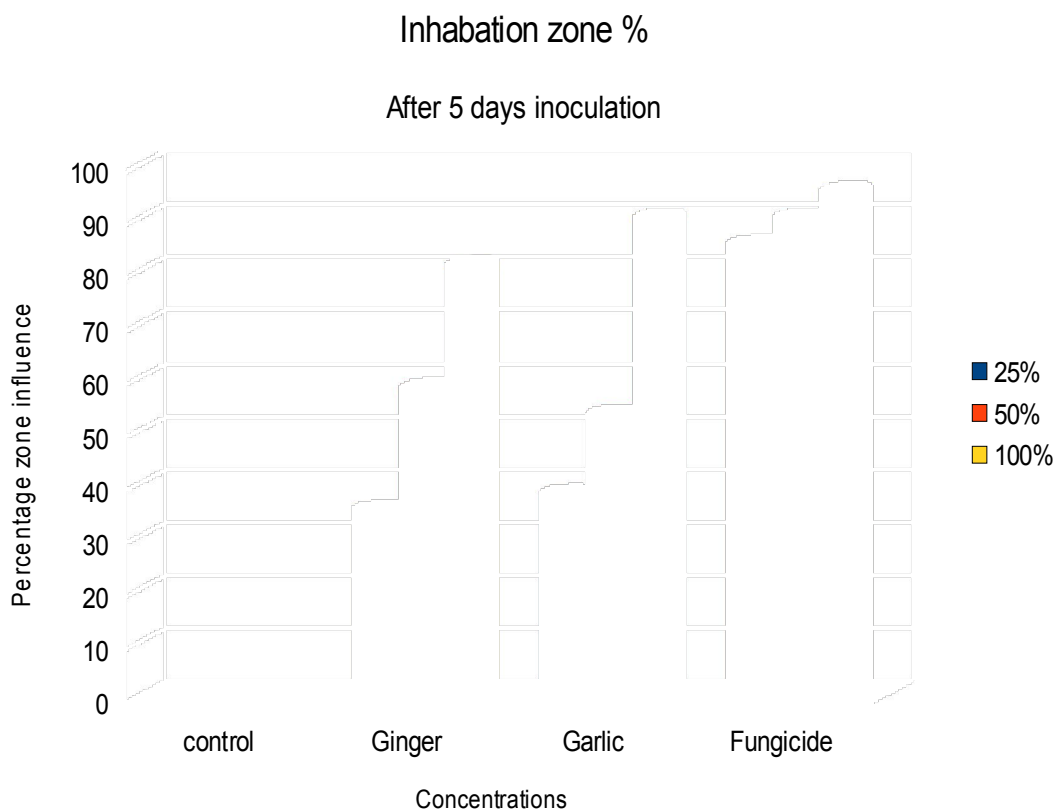
Effect of aqueous extracts of Garlic fruit and Ginger root and Fungicide Revus top on the linear growth of *Fusarium solani* invitro.

After five days from inoculation the results (Table, 3 and Figure, 3) showed that extracts of all the plants tested as well as the fungicide proved to be effective in suppressing the fungal growth.

In fact, all tested concentrations of Garlic, Ginger and fungicide induced a significantly higher inhibition zones percentage against test fungus compared to control (Table, 3). Meanwhile, the Garlic aqueous extract at all concentrations tested (25%, 50% and 100%) give (38.5%, 53.5% and 88.7% percent) respectively exhibited more inhibitory effect than the Ginger aqueous extract (25, 50, and 100%) which gave (35.8%, 58.9% and 81.4%) respectively.

Table, 3: Effect of aqueous extracts of Garlic fruit ,Ginger root and Fungicide Revus top R (56.2ai) EC on the linear growth of *Fusarium solani .f.sp.eumartii invitro*.(5days after inoculation).

Treatments Concs. (%)		Inhibition zone (%)			
		R1	R2	R3	Mean
Garlic	25.0%	35.0 (5.9)	38.6 (6.0)	42.0 (6.6)	38.5 (6.0) c
	50.0%	49.7 (0.0)	52.7 (7.0)	58.0 (7.7)	53.4 (7.0) c
	100 %	85.6 (9.0)	95.7 (9.9)	84.7 (9.0)	88.7 (9.4) b
Ginger	25.0%	40.9 (6.3)	35.7 (5.9)	30.8 (5.6)	35.8 (6.0) c
	50.0%	56.8 (7.8)	64.9 (8.0)	55.0 (7.4)	58.9 (7.7) c
	100 %	85.6 (9.2)	78.0 (8.8)	80.6 (8.9)	81.4 (9.0) b
Rivus top	25.0%	84.0 (9.0)	83.6 (9.0)	87.5 (9.0)	85.4 (9.0) b
	50.0%	86.7 (9.0)	85.8 (9.0)	98.9 (9.9)	90.2 (9.5) a
	100 %	100 (10.0)	95.7 (9.9)	92.0 (9.7)	95.9 (9.8) a
Control		0.00 (0.7)	0.00 (0.7)	0.00 (0.7)	0.00 (0.7) d
C.V			14.07(0.0)		
SE			0.760(0.0)		
LSD			2.060(0.0)		



Any two mean value (s) bearing different superscripts (s) are differingsignificantly ($P < 0.05$).

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

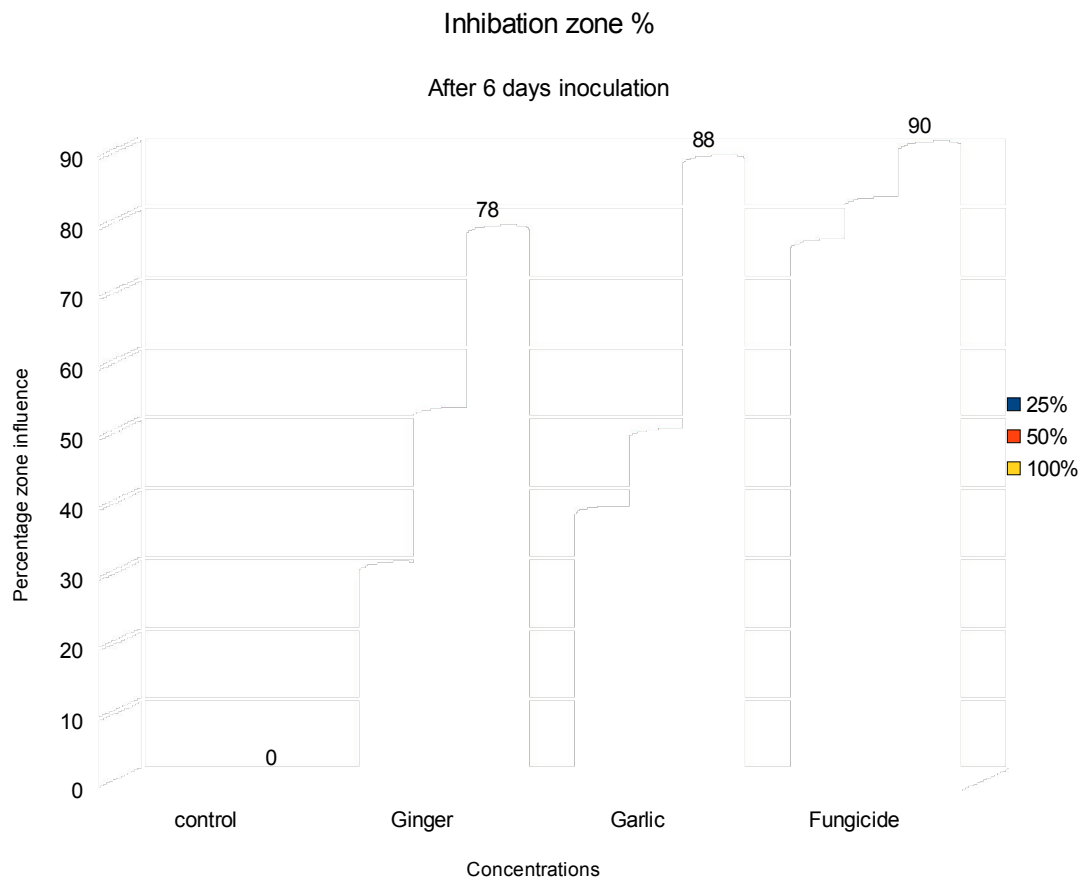
Effect of aqueous extracts of Garlic fruit , Ginger root and Fungicide Revus top on the linear growth of *Fusarium solani* invitro.

After six days from inoculation the results (Table, 4 and Figure, 4) showed that extracts of all the plants tested as well as the fungicide proved to be effective in suppressing the fungal growth. In fact, all tested concentrations of Garlic ,Ginger and fungicide induced a significantly higher inhibition zones percentage against test fungus compared to control (Table, 4). Meanwhile, the Garlic aqueous extract at all concentrations tested (25%, 50% and 100%) give (38.3%, 49% and 88.6% percent) respectively exhibited more inhibitory effect than the Ginger aqueous extract (25, 50, and 100%) which gave (30.8%, 52.8% and 78.9%) respectively.

Obviously, the test organism differs in its response to the different concentrations but on the whole, growth inhibition increased with the concentration. This inhibitory effect from all concentrations was significantly($P < 0.05$) different from control.

Table, 4: Effect of aqueous extracts of Garlic fruit ,Ginger root and Fungicide Revus top R (56.2ai) EC on the linear growth of *Fusarium solani.f.sp.eumartii invitro.*(six days after inoculation).

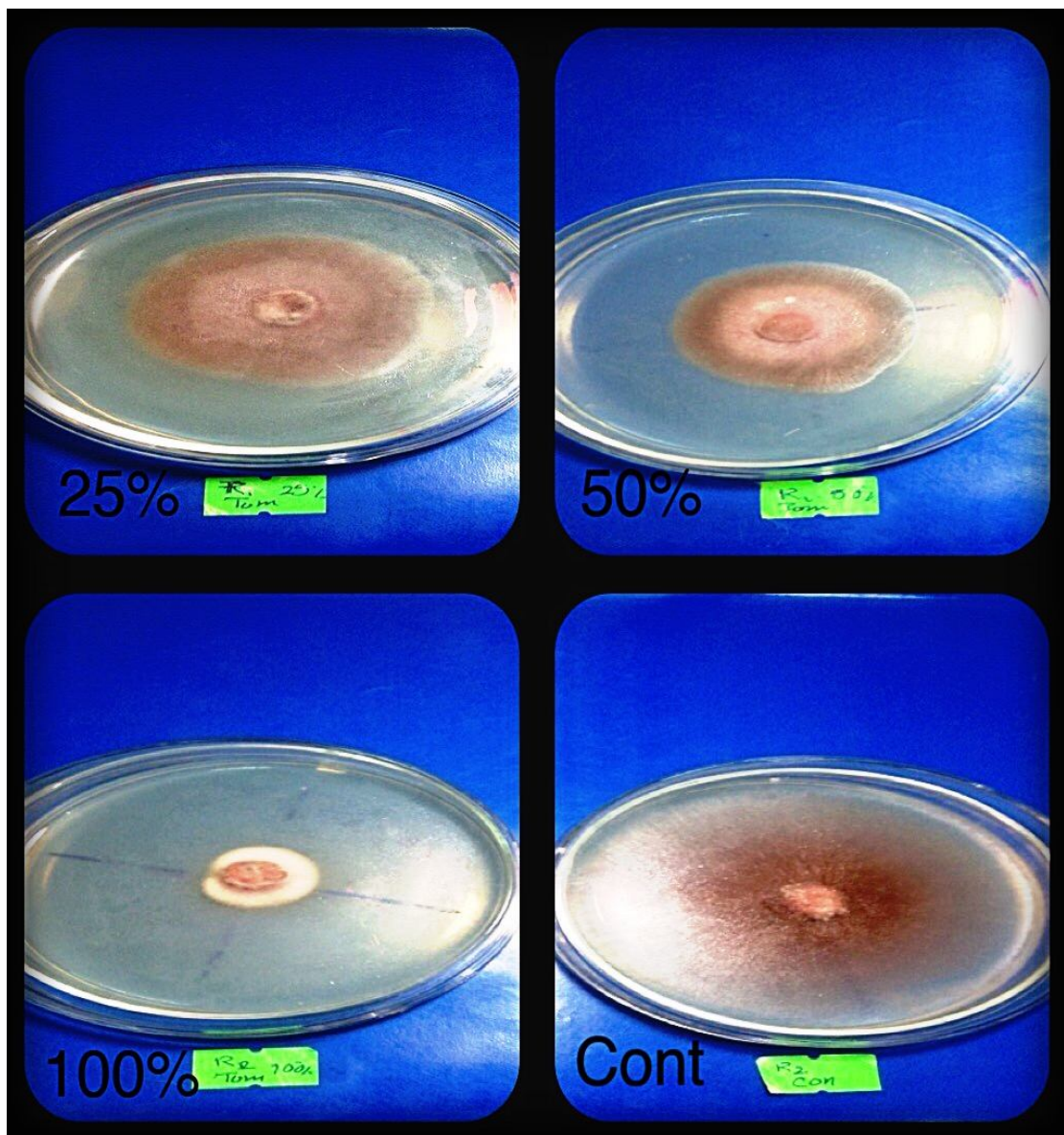
Treatments Concs. (%)		inhibition zone (%).			
		R1	R2	R3	Mean
Garlic	25.0%	38.0 (6.0)	22.6 (4.9)	30.8 (5.6)	38.5 (6.0) b
	50.0%	50.0 (7.0)	50.0 (7.0)	47.0 (6.9)	49.0 (6.8) b
	100 %	86.0 (9.0)	88.0 (9.0)	83.0 (9.0)	88.6 (9.4) a
Ginger	25.0%	35.0 (6.9)	30.9 (5.5)	26.0 (5.0)	30.6 (5.5) b
	50.0%	52.0 (7.0)	53.6 (7.0)	53.0 (7.0)	52.8 (7.3) b
	100 %	73.0 (8.7)	76.0 (8.9)	87.7 (9.0)	78.9 (8.9) b
Revus top	25.0%	78.0 (8.9)	76.0 (8.8)	75.0 (8.7)	76.0 (8.7) b
	50.0%	82.7 (9.0)	78.9 (8.9)	86.9 (8.0)	82.8 (9.5) a
	100 %	92.0 (9.6)	90.0 (9.5)	88.5 (9.4)	90.0 (9.5) a
Control		0.00 (0.7)	0.00 (0.7)	0.00 (0.7)	0.00 (0.7) c
C.V			15.76(0.0)		
SE			0.760(0.0)		
LSD			2.064(0.0)		



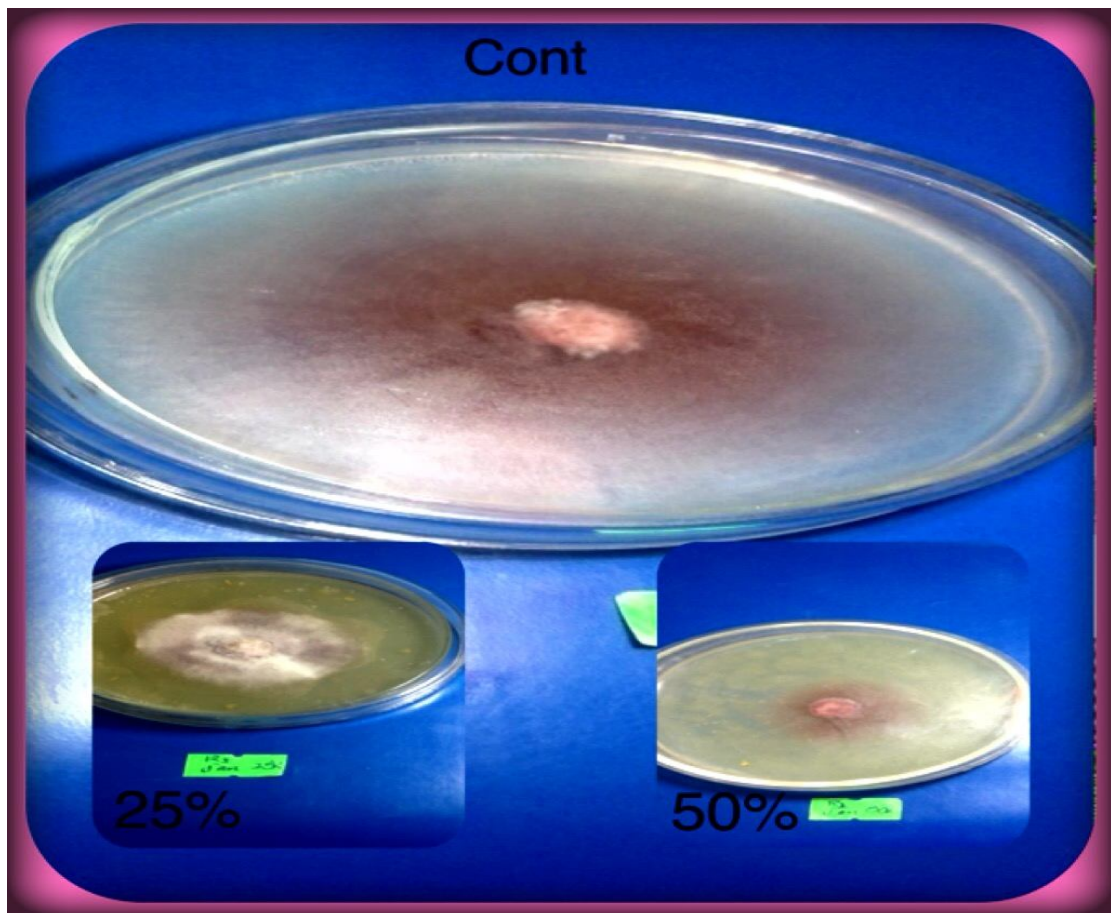
Any two mean value (s) bearing different superscripts (s) are differingsignificantly ($p < 0.05$).

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

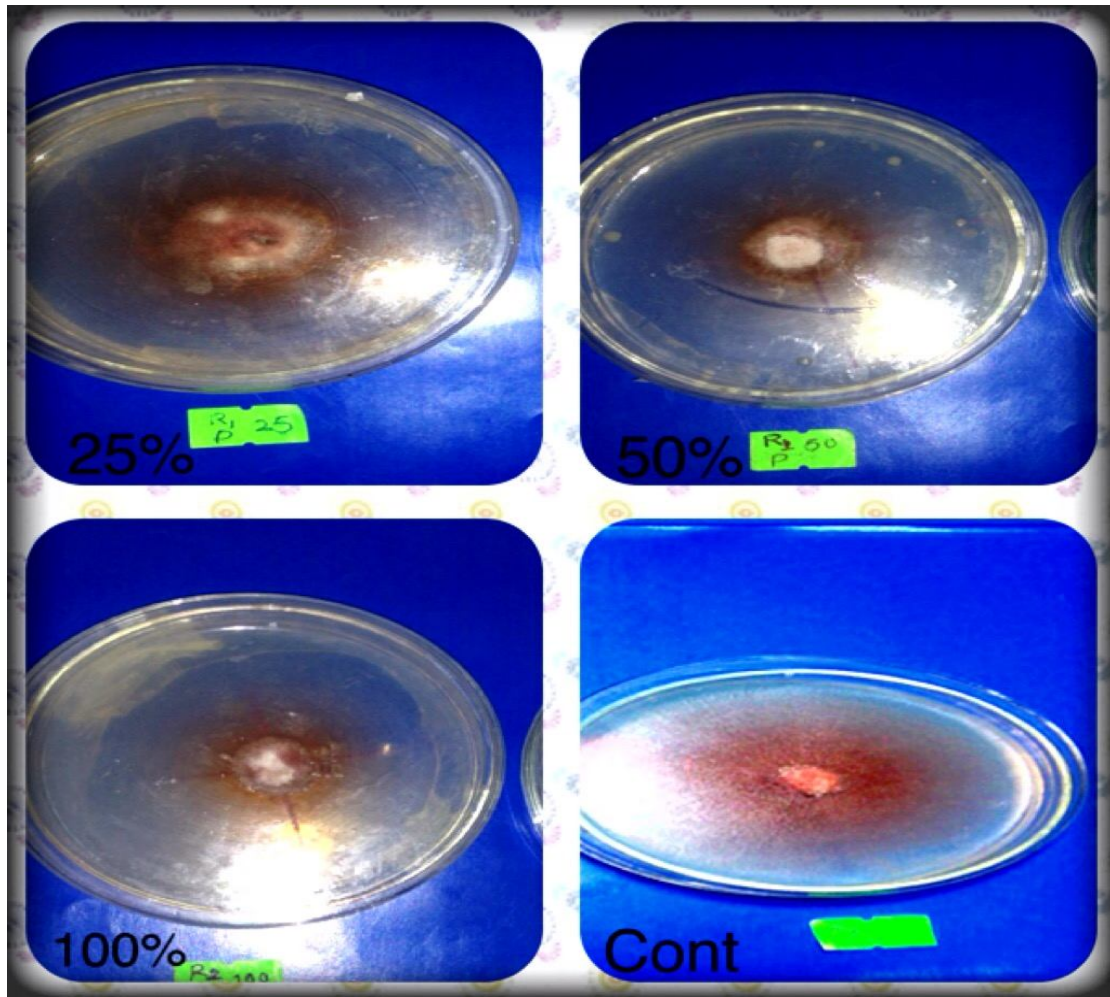
Plat (3) Effect of fruit aqueous extracts of Garlic plant on the growth of *Fusarium solani* invitro.



Plat (4) Effect of root aqueous extracts of Ginger plant on the growth of *Fusarium solani* invitro.



Plat (5) Effect of Revus top EC(56.2 ai) fungicides Control on the growth of *Fusarium solani* invitro.



CHAPTER FIVE

DISCUSSION;

Potato is one of the major vegetable crops grown worldwide as the crop being one of the staple foods of many cultures and civilizations past and presents (FAOSTAT, 2015). One of the threats that limits its production worldwide is Fusarium dry rot which is a worldwide economic disease. In fact, it is a devastating post-harvest problem affecting both seed potatoes and potatoes for human consumption (Nielson, 1981 and FAO, 2008). The disease may cause greater losses of potatoes than any other-post harvest disease. Crop losses attributed to Fusarium dry rot has been estimated to an average of 25% (Powelson *et al.*, 1993). Fusarium species which cause dry rots to potatoes are also important to the consumer because some, Fusarium which cause dry rots also produce mycotoxins, one of such toxins is trichothecene which is an inhibitor of eukaryotic protein synthesis and can pose serious health problem to man and animals (Beremaid *et al.*, 1991).

Generally, management of seed-borne and soil-borne diseases such as Fusarium spp. always had been problematic (Haware, 1992) and (Rao and Balachadran, 2002). Based on the fact that botanical insecticides possess great advantages over synthetic pesticides (Karunyal, 2000; Abdel Moneim, *et al.*, 2009 and Mawda, 2015) in being more environmentally friend and accepted by the majority of the farmers, governmental organizations and decision makers.

The results of this study demonstrated that both aqueous plant extracts (Garlic and Ginger) at all concentrations, exhibited inhibitory effects; consistently throughout the course of the experiment, on the fungal growth of *F. solani* with significantly high inhibition zones percent compared to control. These findings were in agreement with Shrestha and

Tiwari, (2009) as well as Shafique and Shafique, (2012), who assessed the antifungal activity of the crude extracts of six medicinal plants, including garlic, against *Fusarium solani* where they demonstrated that the extracts of all the plant species were found to be effective in inhibiting the mycelia growth of the fungus. Moreover the extract of garlic completely inhibited the mycelia growth of the fungus at the concentration of 40%.

The results on effect of the fungicide on the fungus showed that the fungicide at all concentrations expressed suppressive ability on the growth of *Fusarium solani f.sp.eumartii* with significantly high inhibition zones percent compared to control (Tables 1-4 and Figures 1-4). This results confirmed that reported by Borboru, (1984) Mathre & Johnston, (1995); Ahmad *et al.*, (1996); Baird *et al.*, Nawar, (2007) and Chavan *et al.*, (2009). who demonstrated the efficacy of synthetic fungicides against *F. solani.f.sp.eumartii* where they proved that fungicides tested at different concentrations gave 100 % inhibition of mycelial growth of the fungus. Similar findings on fungicides efficacy against fungi were reported by Abdelgader (2005) and Mohammed (2005).

Fungicides were used @ 10, 50, 100, 500, 1000 and 10,000. Significant effect on colony growth of *F. solani* was observed in all concentration compared to control. Complete inhibition of colony growth of *F. solani* was observed where fungicides viz., Aliette, Benlate and Carbendazim @100 ppm were used whereas Mancozeb, Ridomil, Topsin- M and Vitavax completely inhibited the colony growth at 1000 ppm (Table 1). Benlate has been reported to be most effective for checking the mycelial growth of *F. solani* (Borboru, 1984; Ahmad *et al.*, 1996). Benlate completely inhibited the growth of *F. solani* at 50.0

ppm (Baird *et al.*,1994; Mathre & Johnston, 1995; Nawar, 2007). Carbendazim and Carbendazim +Mancozeb gave 100 % inhibition of mycelial growth of *F. solani* at 0.2 and 0.3% concentrations (Chavan *et al.*, 2009).

The current study also demonstrated that the Garlic extract exhibited more inhibitory effect than that of the Ginger. The high suppressing ability of garlic extract was also presented by Mawda, (2015), which could be attributed to the high concentration of the bioactive inhibiting compound in the Garlic plant fruit than in the Ginger root. Moreover, the data on concentrations from each plant aqueous extract exhibited different inhibitory abilities on fungal growth. The 100 % aqueous extract concentration from the two plants was the most suppressive followed in a descending order by 50% and 25%. Likewise the test organism responded differently to the different concentrations of extracts. This variability in responses was also reported by Aiyelaagbe (2001). In his investigation, he explained that the majority of the studies involving plant extracts demonstrated their inhibitory effects on infectious or harmful microorganisms at variable degree. However, these results were also confirmed by that which obtained by (Reem, (2012); Alhadi ,(2012) and Fayza,(2012).

CONCLUSION

- The use of herbal extracts to control plant diseases is an environment friendly approach and an effective alternative to toxic chemical pesticides. In this study, the effects of plant extracts and fungicide Revus top and their efficiencies against Fusarium dry rot of potatoes were evaluated in vitro conditions, and quite satisfactory results were obtained.
- The results obtained indicated that the aqueous extracts of garlic bulb and ginger root tested as well as the Fungicide Revus top, exhibited an inhibitory effect on fungal growth of the fungus *Fusarium solani.f.sp.eumartii*, the casual agent of dry rot of potatoes. The inhibitory activity of plant extracts was most likely due to antimicrobial components present in plant extracts. However, the exact chemical compounds and their controlling mechanism to the Fusarium dry rot of potatoes need to be elucidated.
- The Garlic bulb aqueous extract exhibited more inhibitory effect than that of the Ginger.
- The screened concentrations of Garlic and Ginger aqueous extracts differ in their reactions to the test fungus. Likewise, the test organism responded differently to the different concentrations of extracts. This variability in response which expressed by test organism may be used to adjust an optimum dose for controlling Fusarium dry rot in potato.

RECOMMENDATIONS;

Based on the foregoing results the following studies are suggested:

- To further investigate the antimicrobial properties of a group of medicinal plants against targets organism to determine their potentials as botanical pesticides,
- To carry out a phytochemicals analysis of different parts of Garlic plant using different solvents so as to determine the exact (active ingredient) and their controlling mechanism to the fungus in each of these parts.

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APPENDIXS

Appendix 1

Table 1: Randomized Complete Block AOV Table for Inhibition

Source	DF	SS	MS	F	P
REPLICAT	2	0.54100	0.27030		
TRETMENT	3	196.789	65.5964	100.51	0.0000
Error	24	15.6640	0.65270		
<hr/>					
Total	29				

Appendix 2

Table 2: Randomized Complete Block AOV Table for INHIBATIO

Source	DF	SS	MS	F	P
REPLICATI	2	0.789 00	0.39430		
TRETMENT	3	184.255	61.4182	73.66	0.0000
Error	24	20.0110	0.83380		
<hr/>					
Total	29	219.4340			

Coefficient of Variation = 16.29%

Appendix 3

Table 3. Randomized Complete Block AOV Table for INHIBATION

Source	DF	SS	MS	F	P
REPLICATION	2	0.02500	0.01230		
TRATMENT	3	204.744	68.2479	57.91	0.0000
Error	24	28.28600	0.178600		

Total 29

Table 4. Randomized Complete Block AOV Table for INHABATION

Source	DF	SS	MS	F	P
REPLICATI	2	0.14100	0.07030		
TRETMENT	3	165.508	55.1694	41.54	0.0000
Error	24	31.8730	0.82800		

Total 29

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

3.1 Equipments.

Needle	laminar
Petri dishes (9cm)	Autoclave
Conical flasks	Incubator
Desiring cylinder	Car
bora	
Sensitive balance	Centrifuge
Filter paper	Microscope
Gloves	Camera
Marker pen	Medical cotton

3.2 Materials.

Potato dextrose agar

Revus top fungicide EC

Infected plant

Garlic fruits

Ginger roots

Distilled water

Sodium hydrochloride (1%)

