Sudan University of Science and Technology

College of Graduate Studies

Effect of Coriander Ethanolic Extract and *Beauveria bassiana* on the Growth of *Fusarium solani* and *pythium digitatium* in Potato Crop

تأثير المستخلص الكحولي لثمار الكسبرة وفطر البيوفيريا علي نمو فطري فيوزيريم سولاني والبيثيوم ديقتاتم علي محصول البطاطس

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BY

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بسم الله الرحمن الرحيم

قال تعالي:

(وَلَسَوْفَ يُعْطِيكَ رَبُّكَ فَتَرْضَى)

صدق الله العظيم

سورة الضحى الآية (5)

Dedication

To my father

To my mother

To my husband

To my teachers

To my friends

To my Brother and Sister

To my sweet hart

Wafaa Rihab Ahmed Ola

With love

Acknowledgements

All my special and unlimited thanks are to Allah, who offered me the health and strength to complete this work.

Full thanks to my supervisor **Dr. Ekhlass Hussein Mohamed Ahamed.**

Also my thanks to all people working in plant protection.

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Abstract

This study was conducted at the laboratory of the Striga Research (Jica), College of Agricultural Studies, Sudan University of Science and Technology 2016.The aim of this study was to evaluate the effects of Coriander ethanol extract, *Beauveria bassiane* and Fungicide Store against the growth of *Fusarium solani* and *pythium digitatium* in culture media Potato Dextrose Agar (PDA) *in vitro* and *in vivo***.** The fungi are an important in causing significant reductions in yield. In the present study, the pathogenic fungi were isolated from infected plant parts. The fungi were identified based on morphological and cultural characters as *Fusarium solani* and *pythium digitatium*. Studies were conducted four concentrations of coriander ethanol extract (100%, 50%, and 25% and 12.5%) in addition to the control treated with distilled water. The results showed that all concentrations exhibited and has an inhibitory effects on the growth of the fungus tested compared to control. The highest inhibition effect was obtained at12.5% (93,86,86 and97) concentration compared by control. Generally, the inhibition zone increase with the decrease in concentration of the extract. In conclusion, this study showed that coriander seeds contain antifungal properties that could be investigated in further studies. Also the use of *Beauveria bassiane* to inhibit the growth *of Fusarium solani* and *pythium digitatium* was gave positive result as biological control compared to control**.** Also the used of fungicide Score to inhibit the growth of *Fusarium solani* was gave the highest inhibition. The chemical control method was found to be the best among all methods used against the fungus. Moreover, botanical and biological control methods were also promising. The overall results suggest the development of an integrated management strategy where chemicals could be combined with botanicals and /or biological methods. Such strategy may decrease rates of the fungicides and consequently cost and increased environmental safety.

ملخص البحث

أجريت هذه الدراسة في مختبر أبحاث البودا بكليّة الدراسات الزراعية، جامعة السودان للعلوم والتكنولوجيا في سنة 2016م الهدف من الدراسة تقييم تأثير المستخلص الكحولي لثمار نبات الكسبرة على فطر الفيوزيريم سولانى وفطر البيثيم ديقتاتم في بيئة بطاطس دكستروز اجار (PDA) مقارنه بالكنترول تحت ظروف المعمل. تسبب هذه الفطريات نقص في الإنتاجية. في هذه الدراسة تم عزل الفطر من أجزاء النبات المصاب، تم التعرف علي الفطر علي أساس الصفات المورفولوجية والمزرعية علي أنه *Fusarium solani* (فيوزويوم سولانى) كما تم عزل فطر البيثيم وتم التعرف عليه . عمل المستخلصات الكحولية من النباتات سهل ومتوفر ويمكن تحضيره بسهولة للسيطرة على أمراض النبات، كما انه غير مكلف، وليس له خطورة على الإنسان وفعّال ولا يؤثر على البيئة. تمت معامله فطري الفيوزيريم والبيثيم باستخدام أربعة تركيزات من المستخلص الكحولي للثمار الكسبرة (100%50%، و25% و12.5%) بالإضافة إلى الكنترول للمقارنة. النتائج التي تم الحصول عليها توضح أن تأثير المستخلصات الكحولية لثمار الكسبرة وفي كل التركيزات أثرت على نمو الفطريات المختبرة مقارنه بالكنترول (67%). تأثير التثبيط الأعلى كان في تركيز 12.5%(.43.,17.,13.و3.) سم.

كلما قل التركيز زادت نسبه التثبيط.. نتيجة لذلك، توضح هذه الدراسة بأنّ ثمار الكسبرة تحتوي على مواد ذات تأثير مضاد لنمو الفطريات، يمكن أن تتحرّى في دراسات أخرى.

كما تمت معامله فطر الفيوزيريم بفطر البيوفيريا باسيانا كمكافحه إحيائية في المعمل و في الحقل وأدت نتائج ايجابيه في السيطرة علي فطري الفيوزيريم والبيثيم كما أثرت علي صفات النمو في محصول البطاطس وكانت ذات اثر ايجابي . وكيميائيا باستخدام المبيد الفطري استور أيضا. وجد ان طريقة المكافحة الكيمائية أفضل من كل الطرف المستخدمة ضد مرض العفن الطري في البطاطس. تعتبر طرق المكافحة الإحيائية واستخدام المستخلصات النباتية أيضا واعده. كل النتائج أدت إلى اقترح تطبيق إستراتيجية المكافحة المتكاملة وهي استخدام المبيدات الكيميائية مع توافق المكافحة باستخدام المستخلصات النباتية أو الطرق الحيوية. هذه الإستراتيجية تؤدي إلى خفض معدل المبيدات الفطرية وبالتالي بتكلفتها وسلامة البيئة.

CHAPTER ONE

INTRODUCTION

The importance of potato (*Solanum tuberosum* L.) has well been noted in several parts of the world, and coming next to major cereals like maize, wheat and rice (Hijmans *et al*., 2000). More than 3 billion people depend on potato as food source (Anonymous, 2014). Globally, more than 300 million hectares of land are under potato cultivation (Anonymous, 2014). In Europe for example, it has gained much recognition as the main tuberous crop in their diets since its introduction in the 1700s (Stark and Love, 2003).
Potato was introduced into the Sudan and grown for the first time in "Tomaniat" village (Al-Gaily area, about 40km north of Khartoum) by a white businessman around 1918 and thereafter it spreads to other parts of Al-Gaily locality (Dr.Siddig M. Elhassan, personal communication).
Khartoum State is the main potato producing area in the country, where over 70% of the crop is produced along the River Nile. Shendi district (River Nile State), on the other hand, has emerged since the last decade as a very + 201important seed- producing area to satisfy some of the local demand from improved Dutch seed potatoes. Jebel Marra, an elevated area in Darfur State is largely dominated by production of the local Zalinge potato stocks. Other potato- producing area in the country includes, Gilo in southern Sudan and limited areas in Kassala and Gezira States .

Fusarium solani is a major wilt pathogen of many economically important crop plants. It is a soil-borne pathogen, which can live in the soil for long periods of time, so rotational cropping is not a useful control method. It can also spread through infected dead plant material, so cleaning up at the end of the season is important (Jones *et.al.,* 1982). 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%. In Michigan, over 50% of seed lots have reported having variable levels of dry rot (Gachango, 2012).

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 (both bacteria) and Trichoderma harzianum (a fungus) in controlling Fusarium dry rot(Warton *et al*,.2013).

Based on the foregoing , this study was undertaken to focus on investigations of various components for management of dry rot of potato caused by *F. solani* such as synthetic fungicides, higher plant extracts and beneficial biological agents in order to formulate promising integrated disease management strategies with following specific objectives:

i) To isolate and identify the causal agent of dry rot and root rot in potato in the main producing areas selected in Sudan

ii) To investigate the efficacy of alternative control measures of dry rot involving the Coriander ethanolic extract, biological agents *Beauveria bassianeum* and fungicide (Store) *in vitro* and *in vivo*.

CHAPTER TWO

LITERATURE REVIEW

2.1 Potato Crop

Potato (*Solanum tuberosum* L.) is one of the most important crops in the world and is planted in 18.2 million ha and total yield reached 314.1 million ton (FAO, 2010). It is most important high nutritive value crop grown in the world (Singh *et. al*., 2004). It comes in the forefront of tuber crops and occupies the fourth position after wheat, sorghum and rice, as an edible and consumed crop in the world. The majority of potato production comes from industrial countries; China, Russia, India, and United States of America with production 72, 63, 23 and 20 million tons/annum, respectively (FAO, 2007).

2.1.1 Classification

Kingdom: Plantae

Phylum : Asterids

Order : Solanales

Family : Solanaceae

Genus : Solanum

Species : Tuberosum

Scientific Name: *Solanum tuberosum*

Egypt is the leading Arab countries in terms of potato production producing about 3.16 million tons/annum followed by Algeria (2.18 million tons) and Morocco (1.6 million tons). Sudan occupies the seventh position with annual production of 0.4 million tons (AOAD, 2006).

In Sudan the area around Khartoum, the capital of the Sudan, benefits from rich water resources (including the Nile and tributaries) and the fertile cultivable land along the River banks is available natural resource. The land suitable for cultivation accounts for about750,000 ha. Of which 11 percent is allocated to urban and per urban agriculture. In Jebal Marra, in the western part of the country, is reported to be the second most important potato production area of Sudan. The Gash Delta area in Kassala Province is often mentioned as a zone of high potential for potato production, though figures on actual production in the area are lacking (Elsir Elamin, 2005).

Potato is one of the major vegetable crops grown worldwide following wheat, maize, and rice, with a production estimates of 368 million tons. 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 (AOSTAT, 2015).

The potato contains vitamins and minerals, as well as an assortment of phytochemicals, such as carotenoids and natural phenols. Chlorogenic acid constitutes up to 90% of the potato tuber natural phenols. Others found in potatoes are 4-O-caffeoylquinic acid (Crypto-Chlorogenic acid), 5-O-caffeoylquinic (Neo-Chlorogenic acid), 3, 4 - dicaffeoylquinic and 3,5 - dicaffeoylquinic acids(Ferretti ,2011). A medium-size 150 g (5.3 oz) potato with the skin provides 27 mg of vitamin C (45% of the Daily Value (DV)), 620 mg of potassium (18% of DV), 0.2 mg vitamin B_6 (10% of DV) and trace amounts of thiamin, riboflavin, folate, niacin, magnesium, phosphorus, iron, and zinc.

The potato is best known for its carbohydrate content (approximately 26 grams in a medium potato). The predominant form of this carbohydrate is starch. A small but significant portion of this starch is resistant to digestion by enzymes in the stomach and small intestine, and so reaches the large

intestine essentially intact. This resistant starch is considered to have similar physiological effects and health benefits as fiber: It provides bulk, offers protection against colon cancer, improves glucose tolerance and insulin sensitivity, lowers plasma cholesterol and triglyceride concentrations, increases satiety, and possibly even reduces fat storage (Raben *et al* 1994). The amount of resistant starch in potatoes depends much on preparation methods. Cooking and then cooling potatoes significantly increases resistant starch. For example, cooked potato starch contains about 7% resistant starch, which increases to about 13% upon cooling (Englyst, 1992).

The storage and cooking method used can significantly affect the nutrient availability of the potato (Madiwale, 2012).

Storage facilities need to be carefully designed to keep the potatoes alive and slow the natural process of decomposition, which involves the breakdown of starch. It is crucial that the storage area is dark, well ventilated and for long-term storage maintained at temperatures near 4 °C (39 °F). For short-term storage before cooking, temperatures of about 7 to 10 °C (45 to 50 °F) are preferred.

On the other hand, temperatures below 4 °C convert potatoes' starch into sugar, which alters their taste and cooking qualities and leads to higher acrylamide levels in the cooked product, especially in deep-fried dishes- the discovery of acrylamides in starchy foods in 2002 has led to many international health concerns as they are believed to be possible carcinogens and their occurrence in cooked foods is currently under study as a possible influence in potential health problems (Tareke, *et al.* 2002).

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, 2003).

Potatoes in Sudan are an important cash crop for small-scale growers, and have the potential to increase incomes in per urban 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.

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, production costs of potatoes are high in comparison with those of other crops; seed potatoes have to be imported and account for more than half of the total production cost of potatoes .This is a major constraint to further expansion of potato production. The estimated total potatoes production in Sudan is about 616,000 tons in a cultivated area of about 88,000 feddan.

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. (Elsir, 2005).

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 6to25% (Gachango *et al*,.2012).
The fungus can persist in the soil for several years. The spores and the mycelium are carried into the soil on tools and in bean straw manure. They may also be splashed by rain or carried by floods. The chlamydospores are the survival structure in the absence of a host plant (Cho *et al*., 2001).

2.2 *Fusarium* dry rot

Fusarium is a large genus of filamentous fungi widely distributed in soil and in association with plants. Most species are harmless saprobes and are relatively abundant members of the soil microbial community. Some species produce mycotoxins in cereal crops that can affect human and animal health if they enter the food chain. The main toxins produced by this *Fusarium* specie are fumonisins and trichothecenes. (Howard, 2003)

2.2.1 Classification

Kingdom: Fungi

Subkingdom: dikarya

Phylum: Ascomycota

Subphylum: Pezizomycotin

Class: Sordaiomycetes

Order: Hypocrites

Family: Nectraceae

Genus: *Fusarium*

Species : Solani

by (Desjardins, 2006)

2.2.2 Biology

In solid media culture, such as potato dextrose agar (PDA), the different special forms of *F. solani* can have varying appearances. In general, the aerial mycelium first appears white, and then may change to a variety of colors - ranging from violet to dark purple according to the strain (or special form) of

F. solani. If sporodochia are abundant, the culture may appear cream or orange in color (Smith, *et.al.*, 1988).

2.2.3 Symptoms

Generally produces symptoms such as wilting, chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting, and damping-off. The most important of these is vascular wilt (Ramsamy, *et.al.*, 1996)

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 (Agrios, 1988, Smith, *et.al.*, 1988).

2.2.4 Disease cycle

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

The fungus can persist in the soil for several years. The spores and the mycelium are carried into the soil on tools and in bean straw manure. They may also be splashed by rain or carried by floods. The chlamydospores are the survival structure in the absence of a host plant. (Cho, *et.al.* 2001).

2.2.5 Hosts Rang

The fungal pathogen *Fusarium solani* 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 (Koenning, 2001).

2.2.6 Environment

As previously stated *F. solani* is a common soil saprophyte that infects a wide host range of plant species around the world. It has the ability to survive in most soil- arctic, tropical, desert, cultivated and non-cultivated (Snyder, *et.al.,* 1940) though *Fusarium* spp. may be found in many places and environments, development of the disease is favored by high temperatures and warm moist soils. The optimum temperature for growth on artificial media is between 25-30°C, and the optimum soil temperature for root infection is 30°C or above (Goss Russ,1936).

2.2.7 Importance

Fusarium solani is so widespread; it is a significant problem in many crops. It is economically damaging to the banana industry, and the threat of more virulent strains or mutations to damage previously resistant crops is of major concern. *Fusarium. solani* also causes damage to many crops from the *Solanaceae* family, including potato, tomato, and pepper. Other commercially important plants affected include basil, beans, carnation, chrysanthemum, peas, and watermelon. Woody ornamentals are infected, but are usually not killed by *Fusarium* wilt alone. Palms, (Dreistadt, *et. al.*, 2004)

2.2.8 Management

Fusarium. solani is a major wilt pathogen of many economically important crop plants. It is a soil-borne pathogen, which can live in the soil for long periods of time, so rotational cropping is not a useful control method. It can also spread through infected dead plant material, so cleaning up at the end of the season is important (Jones *et.al.,* 1982).

One of the control methods is to improve soil conditions because *Fusarium* spp spreads faster through soils that have high moisture and bad drainage.

Other control methods include planting resistant varieties, removing infected plant tissue to prevent over win (Smith, *et. al.*, 1988).

Tering of the disease, using soil and systemic fungicides to eradicate the disease from the soil, flood fallowing, and using clean seeds each year. Applying fungicides depends on the field environment (Booth, 1971).

Fungticides were considered indispensable for sustainable agriculture production, in addition to their role in the protection of human health especially in the tropics (Kiran, *et. al.*, 2006). Meanwhile, the increasing and irrational use of synthetic pesticides has become a source of great concern because of their possible effect on human health and non-target components of the environment (Okigbo, 2004 and Carvalho, 2004). This concern is heightened by the non-specificity and high toxicity of some pesticides and development of resistant strains of microorganisms against other ones. The foregoing has initiated the exploration of safe alternate antimicrobial agents .

Accordingly, increasing efforts have been primary directed towards minimizing pesticides risks and residues in the environment through ecologically sound innovative measures of diseases control (Guddewar, *et.al.*, 1999).

Recently, the uses of natural products for crop protection were greatly emphasized by scientists in everywhere. There are unlimited examples and studies confirmed that higher plants do contain a wide variety of compounds with very good potential for plant diseases control. An example of leaf extracts of Neem tree (*Azadirachta indica*) and chinaberry where reported by Hassanein, *et al.*, (2008) to inhibit *Alternaria solani, F. oxysporum, and, f. sp*. lycopersici, the pathogens of early blight and wilt diseases of tomato.

Likewise, Mint (*Mentha spicata*), Ryhan (*Ocimum basilicum*), and Maharab (*Cymbopogon schoenanthus* Poximus) were tested to control sooty canker pathogen.

Extract from garlic followed by Henna (*Lowsoniai nermis*) leaf extract was reported to control minimum mycelia growth of *Pythium aphanidermatum* (Shenoi, *et.al.*, 1998).

Jatropha curcas L under study are becoming a potential source of natural pesticides. The oil and aqueous extract from oil has potential use as an insecticide, for instance, it has been used in the control of pests of pulses, potato and corn (Kaushik and Kumar, 2004). Medicinal plants have become the focus of intense study in terms of validation of their traditional uses, and then it can use as a natural pesticides. These pesticides are generally more selective in their action, economically feasible and less harmful to the environment than synthetic chemicals (Zhonghua and Michailides, 2005).

Currently, control of plant pathogens requires employment of alternative techniques because traditional handling with synthetic chemicals has caused various problems such as toxicity to users and impairment of beneficial organisms (Anderson, *et al.*, 2003). Another important aspect is that pathogenic organisms have generated resistance to the active ingredient of some synthetic fungicides in response to selection pressure due to high dose and continuous applications, causing great economic losses.

However, natural products proved to be economical and efficient alternative for disease control since it does not affect environment and their residues are easy to degrade (Wilson, *et. al.*, 1995).

2.3 *Pythium spp*

The genus *Pythium* belongs to the family Pythiaceae.

2.3.1 Classification

Family: Pythiaceae,

Order : Pythiales,

Class : Oomycetes,

Phylum: Oomycota

Kingdom Chromista

(Kirk *et al.*,2008).

Pythium species are fungal microorganisms with a filamentous vegetative body called a mycelium. The mycelium of *Pythium* species is colorless, sometimes lustrous, and occasionally slightly yellowish or a grayish lilac (Owen-Going *et al*., 2008). The mycelia in *Pythium* species branch out apically at right angles to form structures known as hyphae. These hyphae are hyaline, with the main hyphae being mostly 5-7 µm wide, occasionally reaching a width of up to 10 µm. Cross septa are lacking except in old, mostly empty hyphae or where the cross septa delimit the hyphae's reproductive organs (Van der Plaats-Niterink, 1981). Protoplasmatic streaming is often clearly visible in young hyphae. According to Postma *et al*., (2009), hyphal walls are composed of 80-90% polysaccharides, mainly ß1-6 linked glucans and ß1-3 and ß1-4 (cellulose). It should be noted that *Pythium* spp. do not contain chitin or chitosan in the hyphal walls, but that they do contain protein and lipid at levels varying from 3-8% and from 1-3%, respectively (Postma *et al*, 2009). Pathogenic *Pythium* spp. may produce hyphae with swollen digitate regions, called appressoria, which enable the fungus to attach and penetrate the host cells (Lévesque *et al*., 2004).

Pythium spp. can reproduce both asexually and sexually. Asexual reproduction takes place through the zoosporangia and zoospores. In *Pythium* the zoospores are not formed in the sporangium itself but in a vesicle

outside it (Stanghellini *et al.*, 1971). The sporangium is separated from the rest of the mycelium by a cross wall. The development of a tube can be observed on the sporangium. The undifferentiated content of the sporangium moves through this tube and forms a vesicle at its end. At this level of development, the zoospores are delimited and start moving (Stanghellini *et al*., 1971). After 10 to 20 minutes, the wall of the vesicle disappears and the zoospores swim away in divergent directions. Zoospores are only liberated under wet conditions. Production of sporangia or hyphal swellings can be stimulated by Mg, K, and Ca ions (Postma *et al*., 2009). Exudates of roots and germinating seeds have a stimulatory effect on the germination of sporangia and on mycelial growth (Stanghellini *et al*., 1971). Sexual reproduction in *Pythium* spp. takes place through the oogonia and antheridia. The female organs, the oogonia, are spherical to limoniform and are intercalary or terminal. The oogonial wall can be smooth or ornamented with projections. The antheridia, the male organs, consist of an antheridial cell, which can be sessile on a hypha, intercalary, or formed terminally on an antheridial stalk (Postma *et al*., 2009). During sexual reproduction, the antheridial cell touches the oogonium and forms a fertilization tube, which penetrates the oogonium. (Van der Plaats-Niterink, 1981). The antheridia are termed monoclinous if they originate from the oogonial stalk and diclinous if they originate from a different hypha not closely connected with the one subtending the oogonium (Hendrix *et al*., 1969). After fertilization, the oogonial content forms a zygote, this evolves into an oospore. Only in rare cases is more than one oospore produced inside an oogonium. The oospore wall is smooth, except in *Pythium dictyosporum* where oospores are reticulate (Van der Plaats-Niterink, 1981). Some stimulatory effects of Ca, Mg, K, Zn and Mn ions on growth and reproduction by oospores have been identified (Hsu *et al*., 1975). Sterols (chlolestorol, ß-sitosterol, etc.) represent important factors in the sexual propagation process. Sterols stimulate growth and reproduction by oospores

and allow the survival of these structures at high temperatures. Such temperatures make the cell membranes of the oospores less permeable to antifungal constituents (Pystina, 1974). After maturation of the oospore, a dormant phase is usually necessary, before germination takes place. At germination, the oospore is converted into a thin-walled structure, which produces a germ tube (Lumsden *et al*., 1987). Oospore germination consists of two stages: first, the absorption of the endospore, depending on an exogenous calcium supply (Lévesque *et al*., 2004), and secondly germ tube formation, which depends on the presence of exogenous carbohydrate sources (Stanghellini *et al*., 1973).

2.3.2 Morphological characteristics used to identify the *Pythium* genus

In the past, the morphological characteristics and size of each of the structures of *Pythium* have been used as the criteria to identify species within this genus.

The major morphological criteria for *Pythium* species identification are based on qualitative characteristics that may vary depending on the culture conditions and the isolate tested (Dick, 2001). Differences in the value attributed to each characteristic have resulted in a confusing taxonomic system for the *Pythium* species (Uzuhashi *et al*., 2010). As a consequence, a more relevant approach to identifying *Pythium* species would be to combine traditional morphological characterization with molecular analyses (Kageyama *et al*., 2003).

2.3.3 Ecology of *Pythium* spp.

Pythium species can be found in various ecological areas such as soils in arable land, pastures, forests, nurseries, and marshes, and in water (Van der Plaats-Niterink, 1981). In general, soil temperature can affect spore germination, germ tube growth and zoospore discharge (Tedla *et al*., 1992). However, each *Pythium* species has its specific optimal development

conditions. Forexample; *P. ultimum* and *P. dissotocum* inhabit cool (10-15 °C) and wet soil as saprophytes on crop residues. Other *Pythium* root rots, such as those caused by *P. aphanidermatum*, *P. irregulare*, *Pythium sylvaticum* Campbell and *P. myriotylum* occur in warm (25-36 °C) and wet soil (Owen-Going *et al*., 2008).

Pythium species have been recovered in soils with a pH ranging from 3.6 to 7.2 (Martin *et al*., 1999). However, in the same study, *Pythium* spp. populations were found to be higher in soils with a pH ranging from 6.8 to 7.2 and lower in soils with a pH ranging from 3.6 to 5.5 (Martin *et al*., 1999). The pH of soil influences some phases of the *Pythium* species life cycle, such as the formation of oospores and sporangia. Alkaline soils (above a pH of 7) favor the growth of *Pythium* species (Lumsden *et al*., 1987). *Pythium* species are more abundant in cultivated than in uncultivated soils: cultivation and incorporation of plant residues into the soil tend to create favorable conditions for the faster decomposition of organic matter, and thus of the availability of fungal food in that environment (Hendrix *et al*., 1970). However, some *Pythium* species are mycoparasites. For example, *P. oligandrum* is nonpathogenic on 12 species of crops from six families, including sugar beet, cucumber, wheat, peas, nephrolepis and common beans (Dušková, 1995; Wulff *et al*., 1998). *Pythium oligandrum* does not attack the tissues of these crops but occurs on the root surface, predominantly in the regions of the hypocotyl (taproot), together with plant pathogenic fungi. *Pythium oligandrum* utilizes the root exudates and fungus hyphae on the root surface, including those of the plant pathogens, for its own nutrition (Brožová, 2002).

2.3.4 *Pythium* root rot control methods

2.3.4.1 Cultural practices

Certain cultural practices have been observed to reduce the severity and incidence of root rot diseases.(Rosado *et al*. 1985) found that the incidence of *Fusarium* spp., *Pythium* spp. and *Rhizoctonia solani* reduced when maize was included in rotation with beans.

Deep plowing and the use of raised ridges to grow beans has been found to reduce root rots favored by high moisture, such as *Rhizoctonia* root rot, southern blight, *Fusarium* root rot and *Pythium* root rot (CIAT, 1995). This is because ridging and deep tillage increase aeration and drainage, creating less favorable conditions for disease development. The application of organic soil amendments is also known to reduce root rot diseases (Voland *et al*., 1994). It has been shown that incorporating *Leucaena* spp. leaves and twigs of *Calliandra magrantha* and *Sesbania*as green manure two weeks before planting reduces plant mortality and increases bean grain yield (Buruchara, 1991; Buruchara *et al*., 1993).

It appears therefore that the approach most likely to be effective in the management of root rots of beans is one of integrated control. In Africa, the integration of organic amendments, raised beds and resistant varieties of beans has been shown to be advantageous over the use of single components in controlling the severity of root rots and crop yield (Buruchara *et al*., 1993; CIAT, 1995).

2.3.4.2 Chemical control of *Pythium* spp.

Once introduced into the soil, *Pythium* spp. may persist for many years through resistance structures such as oospores, zoospores and sporangia (Onokpise *et al*., 1999). In these conditions, applying chemical treatments to kill the pathogen may be an efficient method. There are many specific

fungicides such as benomyl, captafol, captan, carboxin, metalaxyl, propamocarb hydrochloride and etridiazole, which have already proven to be efficient in controlling *Pythium* root rot diseases on beans. However, some fungicides, such as benomyl, are only active on growing mycelium, but not during the resting stage of the mycelium. In the same context, soil fumigants such as methyl bromide, chloropicrin and Vorlex are highly effective biocides that kill *Pythium* agents (Abawi *et al*., 2006). In Latin America and Africa, one of the safest and most economical uses of chemicals to control *Pythium* pathogens consists of coating the seeds of crops. This usually results in effective protection of seeds and young seedlings for about 2 to 3 weeks after sowing (Abawi *et al*., 2006; Schwartz *et al*., 2007). However, given the conditions prevailing in diverse developing countries such as those in Eastern and Central Africa, poor farmers cannot easily afford to apply a chemical control. Moreover, the large scale use of chemical treatment could constitute a source of soil and water contamination, while at the same time exposing poorly educated farmers to health risks related to handling chemical pesticides. Therefore, the one use of chemical control that could be applied in the context of developing countries – *i.e.* chemically coating the seeds of crops in order to protect them against *Pythium* pathogens – cannot be considered as sustainable in bean production by poor farmers in most of those countries.

2.3.4.3 Biological control of *Pythium* spp.

Biological control of soil-borne diseases is particularly complex because the pathogens occur in a dynamic environment at the rhizosphere interface. The rhizosphere is typified by intense microbial activity involving firstly, a high population of microorganisms, and secondly, a rapid change in pH, in salt concentrations, and in osmotic and water potential (Handelsman *et al*., 1996). Microorganisms indigenous to the rhizosphere are ideal for biological control,

since the rhizosphere provides a first-line defense for roots against attacks by plant pathogens (Weller, 1988). Microorganisms can protect the plant from fungal attacks through the production of antifungal metabolites, competition with the pathogen for nutrients, niche exclusion, parasitism or lysis of the pathogen, or through induction of plant resistance mechanisms (Whipps, 1993). Beneficial microorganisms of interest for biological control of plant pathogenic *Pythium* spp. have been identified among fungi and bacteria. Isolates of *Trichoderma* spp. and *Gliocladium* spp. are antagonists of *Pythium*-induced soil-borne diseases and several strains are already commercially available for the biological control of *Pythium* root rots (Howell *et al*., 1993; Fravel, 2005). Various actinomycete species including Streptomyces, Actinoplanes and Micromonospora have the potential to inhibit *Pythium coloratum* cavity-spot on carrots (El-Tarabily *et al*., 1997). Other bacteria effective against *Pythium* are found in various genera including Enterobacter, Erwinia, Bacillus, Burkholderia, Stenotrophomonas, and Rhizobium but the most extensively studied group of bacterial biological control agents is *Pseudomonas* spp. (Chin-Woeng *et al*., 2003; Bardin *et al*., 2004). Competition for organic carbon and iron is one of the mechanisms through which some biocontrol agents suppress *Pythium* spp. (Hoitink *et al*., 1999). Sensitivity of *Pythium* spp. to competition and antagonism during its saprophytic phase of growth is one of the key factors in managing *Pythium* diseases through biological control (Martin *et al*., 1999). In contrast to this view, it is commonly known that *Pythium* spp. propagules germinate rapidly in response to seed or root exudates and quickly infect seeds or roots, and this complicates the application of biological control (Whipps *et al*., 2001). It is, therefore, of great importance that the activity of the biological control agent coincides with the period of host susceptibility and it should persist as long as the plant remains susceptible. Insufficient survival of the antagonists may lead to inadequate or partial control of the

pathogen. From a field experiment conducted in Western Kenya, it was concluded that one approach to addressing this limitation is the introduction of a food base, such as compost, which supports the activity of antagonists but does not stimulate the activity of the pathogen (Otsyula *et al*., 1998; Hoitink *et al*., 1999). However, the compost must be free of *Pythium* root rot pathogens in order to increase the chance of effectively controlling *Pythium* root rot diseases (Martin *et al*., 1985).

2.4 Coriander

2.4.1 Scientific classification:

Kingdom: Plantae

Order: Apiales

Family: Apiaceae

Genus: *Coriandrum*

Species: *C. sativum*

Binomial name: *Coriandrum sativum*

(Ramcharan, *C. (1999).*)

2.4.1.1 Plant Description

Coriander (*Coriandrum sativum*), also known as cilantro, or Chinese parsley is an annual herb in the family Apiaceae and, according to the climatic conditions, is cultivated as a summer or winter annual crop. At flowering, the glabrous plants can reach heights between (0.20-1.4m). The germination is epigaeal and the plant has a tap root. The stem is more or less erect and sympodial, monochasial-branched, sometimes with several side branches at the basal node; each branch finishes with an inflorescence. The color of the more or less ribbed stem is green and sometimes turns to red or violet during the flowering period. They wither before the first fruits are ripe starting from

the basal leaves. The inflorescence is a compound umbel, Sometimes there are one or two linear bracts (Diederichsen, Axel. 1996).

2.4.1.2 Distribution

A native of the Mediterranean region there for several thousand years, now cultivated in tropical Asia (India, Malaysia, Thailand, and china), the Middle East and Brazil. Coriander was brought to the British colonies in North America in 1670, and was one of the first spices cultivated by early settlers. (Aggarwal, and Kunnumakkara, 2009) and (Platte, 1962).

2.4.1.3 Ecology and agronomy

The best descriptions of the agronomic needs of coriander cultivation for use of the fruits are given by (Luk'janov and Reznikov, 1976). For Central Europe, the advice of (Heeger, 1989) is useful, and some more recent hints on cultivation. Indian conditions have been reviewed by (Singh and Gangwar, 1991). Cultivation for production of coriander fruits is only possible if the sum of average temperatures of the days during the vegetation period is at least 1700-1800°C (Luk'janov, and Reznikov, 1976).

The introduction of coriander, even under irrigation, has recently been reported for Argentina (Luayza, *et al*. 1995). High temperatures and sunny weather during the flowering period will favor the yield of fruits and the essential oil content.

2.4.1.4 Areas of production and consumption

World production of coriander fruits is difficult to estimate, since official statistics seldom contain figures relating to this crop. A considerable quantity of coriander is grown in home gardens or on a small scale, and is not recorded in any statistics. Taking the different observations on this subject into account, the world-wide production of coriander may be estimated at

approximately 550 000 ha annually. The yearly production of coriander fruits may be estimated at about 600 000 t.

The tropical climate is unfavorable for ripening of the fruits, and coriander is only cultivated for the use of the fruits in mountainous areas of the tropics. The plant is sometimes cultivated for use as a vegetable in tropical areas, e.g. in Cuba.

The International Trade Centre (1986) estimated that in 1986, the world production of coriander essential oil was 90-100 t/ year. The main producer of coriander for export has always been the former Soviet Union, and (Luk'janov and Reznikov in 1976) reported that 98% of the world's coriander essential oil was produced by the Soviet Union. The same authors estimated the total global area of land in coriander production to be 320 000 ha.

2.4.1.5 Chemical composition of the fruits

The essential oil content is of greatest importance, there are accessions that have almost no essential oil and others with up to 2.60% of essential oil in the air-dried fruits. The extremely leafy types from Syria have very low essential oil content in the fruits. Despite this, the essential oil content is positively correlated with the foliation of the plant.

The taste of the green leaves of the plant was more aromatic in the accessions that had high essential oil content in the fruits. The Georgian types had leaves with a very spicy taste. The Syrian types must have been subject to a selection towards plants with a mild taste more suited to use in salads than as a spice. The Ethiopian accessions show the same tendency as the Syrian, but their flavor is more aromatic.

Usually, the plants with low foliation and large fruits have allowed essential oil content. The Indian group with the lengthened fruits also belongs in this category.

2.4.1.6 The essential and fatty oils of the fruits

The uses of coriander fruits are related to their chemical composition. The general composition of the fruits is presented in Table 2. The most important constituents are the essential oil and the fatty oil. The essential oil content of the weight of ripe and dried fruits of coriander vary between 0.03 and 2.6%, and the content of fatty oil varies between 9.9 and 27.7% that reported by (Diederichsen, Axel. 1996).

2.4.1.7 Medical uses

Coriander has been used in medicine for thousands of years (Mathias, 1994). The first medicinal uses of the plant were reported by the ancient Egyptians. General references to coriander's medical uses are also found in classical Greek and Latin literature (Manniche, 1989), and instructions to cultivate coriander are contained in the German emperor Charlemagne's decree 'Capitulare de villis' in 1812. The coriander fruits are believed to aid digestion. Many other fruits of umbel liferous plants have been used in medicine since antiquity (French, 1971) as they also affect the digestive system and some act as an aphrodisiac. Some of these, such as hemlock (*Conium maculatum L.*), are poisonous. Coriander is also used externally to treat ulcers and rheumatism; these and several other medicinal uses are recorded by (Diederichsen, Axel. 1996).

The fruits need to be soaked in wine or in vinegar overnight before being re-dried, in order to remove chemical compounds contained in the fresh fruits, which cause dizziness. These are mentioned in the older references (Linnaeus, 1780). Fruits thus treated were used for medicinal purposes, and also to treat

halitosis. Today, the plant is still sometimes used for these purposes in folk medicine. The medical uses of coriander in the modern era are described by (Diederichsen, *et al*. 1996). In India, the fruits are considered carminative, diuretic, tonic, and stomachic, ant bilious, refrigerant and aphrodisiac (Diederichsen, Axel. 1996).

Plate 1. Coriader

2.5 *Beauveria bassiane* (Balsamo)Vuillemin (Deuteromyca)

Beauveria bassiane is a soil borne necrotrophic parasite that has been documented as an entomopathogenic fungus for centuries. The fungus was first discovered around 900 AD in silkworms found in Japan (Boucais and Pendland, 1998). A fungus similar to the present-day description of *B.bassiana* was also found in a worker ant buried in amber and estimated to be 25 million years old (Poinar and Thomas, 1984). Antiseptic properties have also been noted for *B.bassiana* and it has been used for the treatment of sore throats and wounds (Boucais and Pendland, 1998). In 1834, Italian scientist Antonio Bassi de Lodi demonstrated that the white muscardine disease of silkworms was caused by a fungus. (Boucais and Pendland, 1998). The fungus was originally named *Botrytis paradoxa* by Balsamo, but it was later changed to *Botrytis bassiana* in honor of Bassi. The genus was changed to *Beauveria* in 1912 by Vuillemin, which led to the current binomial, *Beauveria bassiana* (Balsamo) Vuillemin (Steinhaus, 1949; 1975;

Alexopoulos *et al*., 1996; Boucias and Pendland, 1998). A relatively new finding is that *Beauveria bassiana* can grow endophytically in plants. At this time, it is unknown whether the responses of tomatoes to *Beauveria bassiana* resemble systemic acquired resistance or induced systemic resistance or if it more closely resembles true plant endophytes like grass-*Neotyphodium* interactions.

Currently, an isolate of the fungus is used as a biocontrol agent of insect pests and is marketed under the names Botani Gard, Mycotrol, and Naturalis. Although these formulations are routinely sprayed on plants, the degree of host infection and subsequent endophytism is unknown.

Mycelium of the fungus is white, yielding the common name white muscardine fungus. Conidia are hyaline, dry, and globose to oval shaped; they are found on the main hyphal branches or as an extension of the conidiophore. The infectious conidia may be found on the flask-shaped conidiophore in clusters, whirls, or singly, in an apical zigzag formation known as a rachis. The sexual stage (teleomorph) of *B. bassiana* is *Cordyceps* sp. Filiform, multiseptate ascospores are found in the asci of the perithecium, or reproductive structure, of the teleomorph (Boucais and Pendland, 1998). *Beauveria bassiana* can infect insects of most orders and has world-wide distribution, unlike most other deuteromycetes. The fungus colonizes insects with the aid of mycotoxins, such as beauvericin and oosporein, then continues to grow out of the cadaver, forms conidiophores and subsequently releases conidia for dispersal (Steinhaus,

1949; Boucias and Pendland, 1998). Endophyte colonization of plants was first notedwhen corn (*Zea mays* L.) plants were treated with *Beauveria* to control European cornborer (*Ostrinia nubilalis* (Hübner)) (Bing and Lewis, 1991). The fungus colonized the plant when applied to foliage as either a granular formulation or injected as a conidial suspension. *Beauveria bassiana* colonized the xylem vessels (Bing and Lewis, 1992). When movement of the

fungus through the corn plant is monitored using light and electron microscopy, germinating hyphae from a foliar application grew and penetrated the corn leaf surface at random (Wagner and Lewis, 2000). Invasion was achieved primarily through direct penetration of the epidermal cell wall rather than through stomata. Once inside, the cuticular ultra structure of the plant cell wall was noticeably distorted, and growth was observed in the air spaces between the parenchyma cells (Wagner and Lewis, 2000). Hyphal structures were observed in the xylem elements, but primarily following the leaf apoplast away from the point of penetration (Wagner and Lewis, 2000). The observed structures in the xylem vessels indicate the possibility of movement throughout the plant and subsequent protection from insects.

More recent investigations have shown that *B. bassiana* isolate 11-98 (Bb 11-98) endophytically colonizes cotton (Griffin *et al*., 2005), tomato (Leckie, 2002; Ownley *et al*., 2004), and snap bean (Ownley *et al*., unpublished data) when seeds were treated with conidial suspensions of the fungus. *Beauveria* has been isolated from all three plants using selective media (Griffin *et al*., 2005; Ownley *et al*., 2004; Doberski and Tribe 1980). Using polymerase chain reaction (PCR) endophytic growth was confirmed in tomato seedlings grown from Bb 11-98 coated seeds (Leckie, 2002).Seed were coated with Bb 11-98 and grown in medium infested with mycelium of the fungus; subsequent seedlings did not differ from non-coated seed grown in uninfested media in terms of survival, shoot weight, and root weight when not challenged with *Rhizoctonia solani*. However, damping-off was reduced in the Bb 11-98 treated compared to the pathogen control (Seth, 2001). In addition, Bishop (1999) found treatment of ‘Mountain Spring’ seeds with *Beauveria bassiana* suppressed pre emergence damping-off caused by *Rhizoctonia solani*. More recently, when leaf aphid, *Macrosiphum euphorbiae,* fed on tomato foliage treated with conidial suspensions of Bb 11-98, mortality was 29 and 40% at 7 and 10 days after treatment, respectively

(Powell, 2005). When *Beauveria bassiana* 11-98 is applied as a tomato seed treatment, plant stand in *Rhizoctonia solani*-infested media does not differ from plant stand in media that is not infested with the pathogen. (Ownley *et al*., 2000; Ownley *et al*., 2004; Ownley *et al*., un published data). *Beauveria bassiane* has been proven time and again to be an effective entomopathogen. Additionally, the endophytic ability of the fungus also gives it outstanding promise as a biological control against other fungi, bacteria, and viruses, by decreasing competition from plant pathogenic organisms.

Fungicide

SCORE® has a yield boosting effect to enhance its superior crop protection.

Additional brand. ARMURE®, TASPA®, SICO® INSPIRE® Active ingredient Difenoconazole Mode of action Stops the development of fungi by interfering with the biosynthesis of sterols in cell membranes. Long-lasting preventive activity combined with curative and eradicant properties; fast uptake and high translaminar movement. Targets A wide range of leaf spot diseases. Key partner for resistance management program Main crops Can be used on almost any crop. Geography Europe, Asia, Americas, Africa, Oceania Main customer benefits Ideally systemic, SCORE® gives more to growers: it is reliable and economical, giving a high return (Gachotte, D., Husselstein, T., Bard, M., Lcroute, F. & Benveniste, P. 1996).

Plate 2. Fungicide Score

CHAPTER THREE

MATERISLS AND METHODS

This study was conducted at the Jica laboratory, Department of Plant Protection, college of Agricultural studies, Sudan University of Science and Technology during December 2015-January, 2016.The aim of this study was to investigate the antifungal activities of coriander ethanol extract and *Beauvearia bassiane* against the growth of the two fungi *Fusarium solani* and *pythium digitatum*, in culture media under laboratory conditions (*in vitro*) where temperature around 28 ^{o}C and field condition (*in vivo*).

3.1 Isolation and Identification of the fungus Fusarium and Pythium

3.1.1 Collection of samples

A potato tuber showing symptoms of infection was collected from Khartoum Bahre vegetable market.

3.1.2. Isolation methods

Isolation is conduct at the laboratory of the Plant Pathology following steps adapted by Mamatha., (2004), Cleaning of potato tubers thoroughly with fresh water then infect parts cut into discs about 1-2 cm, after that the potato discs dips into Clorox 10% (2min), alcohol (2min) and rinse with sterilize distill water for 2min. the clean discs transferee to Petri-dish containing filter paper to dry, until that time prepared Potato Dextrose Agar (PDA) media and place in Petri –dishes (3 Petri-dishes) then inoculate with the diseases discs under laminar flow cabinet. The Petri dishes tightens with paraffin film and incubated at 28C^{0} for 7 days to allow fungal growth.

3.1.3 Purification of fungus

The growing culture examine under the microscope at 1000 magnification. A serial of sub culturing is doing to obtain pure culture of Fusarium which later use in experiments.

3.2 Source of *Pythium digitatium*

A potato tuber showing symptoms of infection collect from Khartoum vegetable market.

3.2.1 Isolation method of *Pythium digitatium*

Isolation is conduct at the laboratory of the Plant Pathology following steps adapt by Mamatha, (2004) mentioned above

3.2.2 Purification of fungus

The growing culture examine under the microscope at 1000 magnification. A serial of sub culturing is doing to obtain pure culture of pythium which is use in experiments.

3.2.3 Preparation of the Botanical extracts (coriander)

Samples of coriander (*Coriandrum sativum)* obtain from Khartoum vegetable market. Corainder fruit surface-sterilize by immersion in 1% sodium hypochlorite obtain by dilution of the respective amount of commercial bleach solution (NaOCL) for 5 min. Subsequently the coriander fruit thoroughly wash with sterilize distill water then air dire in a lamina flow and store at ambient temperature, till use. After that dry samples grinding using an electric blender (Monelex).

Extracts from seeds of Coriander 100gm of powder dire sample weight and 500ml ethanol add, for hot extraction using Soxleate approuts. solvents for 6 hour at (70°C) with shaking , the extracts filtrate through filter paper under

suction reducing pressure .the filtrate concentrate by using rotary evaporator to remove the solvents, the other concentration obtain by dilute to subsequently 12.5, 25, 50, 100%.The PDA media amend with five milliliters of ethanol extract, 1 and 5%, of each plant extracts individually inoculat with mycelial discs (9 mm diameter) take from the advancing edges of 7 day-old pure cultures of *F. solani* and *P. digitatium*. The control experiment has distilled water instead of plant extracts. The inoculate media incubate at temperature 27±1°C. Three plates of each treatment are use as a replicates. The diameter of the fungal colony measure using a meter rule along two diagonal lines drawn on the reverse side of each Petri plate 7 days after inoculation. Each treatment was replicated three times with four plates per replication (Pandey,. 2007).

3.3 Preparation of fungicide concentrations

100ml of the score 250 EC (Difenoconazole 25%) take into 100 ml volumetric flask complete to the mark using sterilize distill water. 5 ml of recommend fungicide doses (score 250 EC) add to 95 ml of PDA medium mixed well. The prepare media pourer into sterilize glass Petri dishes 2500 ppm fungicide concentrations.

3.4 Test procedure

The antifungal in vitro assays carry out following the modified method of (Okigbo *et al*. 2005) and (Chohan, and Perveen, 2006). Inhibition zone technique is uses in this study to evaluate the effect of each concentration on mycelia linear growth of the fungus. Initially, fresh fungal growth prepare from previously maintained culture of *F.solani* and *P. digitatium*. Prepared PDA media amend with the require concentration from Coriander and fungicide before being solidified in a conical flask of 250 ml, agitate and poure into sterilize 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 serve as control.

One mycelia disc of the fungus was placed in the centre of PDA plates where opposite poles were marked at the back of the plate and incubate at 27±1°C in incubator and radial growth of pathogen measure at 24 hour intervals.

The Petri dishes of each concentration arrange in a complete block design in incubator and incubated at 27±1°C for 3 days. The growths of the fungus are measure and calculate successively after 3, days after inoculation. The effects of each extracts concentration on linear fungal growth calculate as percentage of reduction in diameter of fungal growth. The formula suggested by Vincent (2001). The formula Where: -

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

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 Analysis Procedure

The data Obtained Was Statistically analyzed statistix 8 according to Complete Randomizied Block Design (CRBD) analysis of variance (ANOVA), L.S.D test was used for means separation.

CHAPTER FOUR

RESULT

This study was conducted under laboratory condition of plant protection department, College of Agricultural Studies, Sudan university of science and Technology (During November and December 2015) the aim of this study was to investigate the antifungal activities of Coriander (*Coriander sativum*) Ethanol extracts, *Beauveria basiane* as biological control and fungicides score against *Fusarium solani* and *Pythium digitatium in vitro* and *in vivo*

4.1Identifications of *Fusarium solani* and *Pythium digitatium*

Identification of *Fusarium solani* was performed depending on the cultural characteristic and conidia, hyphae, mycelium and spores shapes as described by Booth (1977)

Plate 3. *Fusarium solani*

Identification of *Pythium digitatium* was performed depending on the cultural characteristic and conidia, hyphae, mycelium and spores shapes as described by (Agrious,.2005)

Plate 4. *Pythium digitatium*

4.2 Effect of coriander ethanol extracts and fungicide on the linear growth of *Fusarium solani* after three days of inoculation *in vitro*

The results (Table1) showed that the ethanol extracts of Coriandera and fungicide exhibited an inhibitory effect on the fungal growth after three days from inoculation. All concentration of coriander ethanol extracts (12.5%, 25%, 50%, 100%) gave the highest inhibition of mycelia growth (88, 79,.81 and 96) respectively. Furthermore, the percentages inhibition of fungal growth was significantly high compared to control. However, the suppressing effect of fungicide was 100% than other treatments.

4.2.1Effect of coriander ethanol extracts and fungicide on the linear growth of *Fusarium solani* after four days of inoculation *in vitro*

Four days after inoculation, all concentrations of ethanol extracts of Coriander demonstrated the significantly highest inhibition zone percent (93, 86, 86 and 97) respectively. Obviously, this inhibitory effect from all concentration was significantly different from control.

4.2.2 Effect of coriander ethanol extracts and fungicide on the linear growth of *Fusarium solani* after five days of inoculation *in vitro.*

Table 1 showed that the ethanol extracts of Coriander exhibited an inhibitory effect on the fungal growth after Five days of incubation. All concentration of

the Coriander extracts (12.5%, 25%, 50% and 100%) gave the highest inhibition of mycelia growth (93, 86, 86 and 97) compared with the control.

In fact, all concentrations of Coriander and fungicide continued inducing a significant inhibition zones percentage against *F.solani* compared to control. Obviously, the *F.solani* differs in its response to the different concentrations.

4.2.3 Effect of coriander ethanol extracts and fungicide on the linear growth of *Fusarium solani* after six days of inoculation *in vitro*

Six days after incubation the results (Table 1) showed that the ethanol extracts of Coriander and fungicide exhibited an inhibitory effect on the fungal growth. All concentrations extracts (12.5%, 25%, 50%, 100%) gave the highest inhibition of mycelia growth (1.06, 1.08, 0.93 and 1.16) compared with the control.

Table 1. Effect of coriander ethanol extract and fungicide on *Fusarium solani* after inoculation *in vitro*

Treatments	Growth of *Fusarium solani* (cm) Days after inoculation			
	3 days	4 days	5 days	6 days
Control (untreated)	1.9 (1.56)a	2.19 (1.64)a	3.70 (1.64)a	3.77 (2.06)a
Coriander 100%	0.43(0.96)b	0.46 (0.97)b	0.66 (0.97)b	0.87 (1.16)b
Coriander 50%	0.17(0.81)bc	0.27(0.86)bc	0.33 (0.86)bc	0.40 (0.93)bc
Coriander 25%	0.13(0.79)bc	0.28 (0.86)bc	0.48 (0.86)bc	0.70 (1.08)b
Coriander 12.5%	0.3(0.88)b	0.40 (0.93)b	0.55 (0.93)b	0.67 (1.06)b
Fungicide(score)	0.00 (0.00)c	0.00 (0.00)c	0 (0.00)c	0.00 (0.00)c
CV%	10.42%	13.14%	10.80%	11.84%
SE+-	0.7	0.8	0.11	0.11
LSD	0.1779	0.2320	0.2179	0.2452

Means in the same column with same letters are not significant at P= 0.05

Values between brackets transferred to ($\sqrt{X + 0.5}$)

Table 2. Effect of coriander ethanol extract and fungicide on linear growth *of Pythium digitatium* after inoculation *in vitro*

Treatment	*Pythium digitatium* Growth(cm) Days after inoculation		
	3days	4days	5days
Control	2.61(1.75)a	2.83 (1.82)a	3.49 (1.99)a
Coriander 100%	0.0 0 (0.70)b	0.00 (0.70)bc	0 (0.70) b
Coriander 50%	0.05 (0.73)b	0.14(0.78)b	0.17 (0.80)b
Coriander 25%	0.00 (0.70)b	0.00 (0.70)bc	0 (0.70)b
Coriander12.5%	0.00 (0.70)b	0.00 (0.70)bc	0 (0.70)b
Fungicide(score)	0 (0.70)b	0 (0.46)c	0 (0.70)b
CV%	6.94%	20.39%	7.91%
SE+-	0.10	0.11	0.12
LSD	0.1125	0.3132	0.1258

Means in the same column with same letters are not significant at p= 0.05

Values between brackets transferred to($\sqrt{X + 0.5}$).

Table 3. Effect of *Beauveria bassiane* on the growth (plant height) of potatoes in the field against *Fusarium solani* and *Pythium digitatium*

Treatments	Plant height (cm)		
	After 20 days	After 30 days	After 40 days
Potato un treatment	15.67(3.997)a	17.83(4.250)a	19.67(4.470)a
Potato + *Beauveria bassiane*	43.5 (3.847)a	14.5 (3.847)a	19 (4.187)a
Potato+ *Fusarium solani*	15.33(3.970)a	17 (4.180)a	18.66(4.377)a
Potato +*Pythium digitatium*	14.33(3.847)a	14.67(3.887)a	17 (4.180)a
Potato+ *Beauveria bassiane*+ *Fusarium solani*	13.33(3.713)a	15.16(3.957)a	16.67(4.140)a
Potato+ *Beauveria bassiane*+ Pythium digitatium	14.66(3.893)a	18.33(4.327)a	18.33(4.327)a
CV%	9.54%	9.77%	8.19%
SE+-	0.08	0.09	0.08
LSD	0.6585	0.7071	0.6239

Means in the same column with same letters are not significant at p= 0.05

Values between brackets transferred to($\sqrt{X + 0.5}$).

Table 4. Effect of *Beauveria bassiane* on potato shoot (fresh and dry) treated by *Fusarium solani* and *Pythium digitatium in vivo* pot.

Treatments	Weight of shoot (g)	
	Fresh	Dry
Potato un treatment	14 (3.723)a	1.7 (1.483)abc
Potato+ Beauveria bassiane	3 (1.857)b	3 (1.857)ab
Potato+ Fusarium solani	13.67(3.743)a	4.17 (2.077)a
Potato +Pythium digitatium	5 (2.303)b	0.87 (1.177)c
Potato+ Beauveria bassiane+ Fusarium solani	8.33(2.873)b	1.17(1.293)bc
Potato+ *Beauveria bassiane*+ Pythium digitatium	7.33(2.2.697)ab	1.03(1.213)c
CV%	25.58%	23.47%
SE+-	0.22	0.11
LSD	1.304	0.6340

Means in the same column with same letters are not significant at P= 0.05

Values between brackets transferred to($\sqrt{X + 0.5}$).

Table 5. Effect of *Beauveria bassiane* on potato root(fresh and dry)treated by *Fusarium solani* and *Pythium digitatium*

Treatments	Weight of root (g)	
	Fresh	Dry
Potato un treatment	14 (3.723)a	1.2 (1.297)a
Potato+ *Beauveria bassiane*	7.33 (2.783)a	1.03(1.223)a
Potato+ *Fusarium solani*	9 (3.067)a	2.16(1.630)a
Potato + *Pythium digitatium*	9.33 (3.003)a	2.3(1.583)a
Potato+ *Beauveria bassiane* + *Fusarium solani*	12.33 (3.537)a	2.17(1.627)a
Potato +*Beauveria bassiane*+ Pythium digitatium	13 (3.600)a	2.53(1.687)a
CV%	24.27%	24.43%
SE+-	0.18	0.08
LSD	1.419	0.6561

Means in the same column with same letters are not significant at P= 0.05

Values between brackets transferred to($\sqrt{X + 0.5}$)

Table 6. Effect of *Beauveria bassiane* potato yield treatedby *Fusarium solani* and *Pythium digitatium*

Treatments	Yield(Kg)
Potato un treatment	4 (2.113)b
Potato+ *Beauveria bassiane*	4.67 (2.257)b
Potato+ *Fusarium solani*	8.67 (3.003)a
Potato +*Pythium digitatium*	3(1.857)b
Potato+ *Beauveria bassiane*+ *Fusarium solani*	4(2.083)b
Potato+ *Beauveria bassiane* +Pythium digitatium	2.67 (1.737)b
CV%	18.34%
SE+-	0.13
LSD	0.7094

Means in the same column with same letters are not significant at p= 0.05

Values between brackets transferred to ($\sqrt{X + 0.5}$).

Table 7. Effect of *Beauveria bassiane* on the growth of potato against *Fusarium solani* and *Pythium digitatium*.

Treatments	Tuber weight(g)
Potato un treatment	45.33 (6.317)a
Potato+ *Beauveria bassiane*	35.33 (5.877)a
Potato+ *Fusarium solani*	29.33 (6.453)a
Potato + *Pythium digitatium*	24.67 (4.960)a
Potato+ *Beauveria bassiane* + *Fusarium solani*	35 (5.750)a
Potato+ *Beauveria bassiane* + *Pythium digitatium*	44 (6.643)a
CV%	30.77%
SE±	0.39
LSD	3.284

Means in the same column with same letters are not significant at p= 0.05

Values between brackets transferred to($\sqrt{X + 0.5}$).

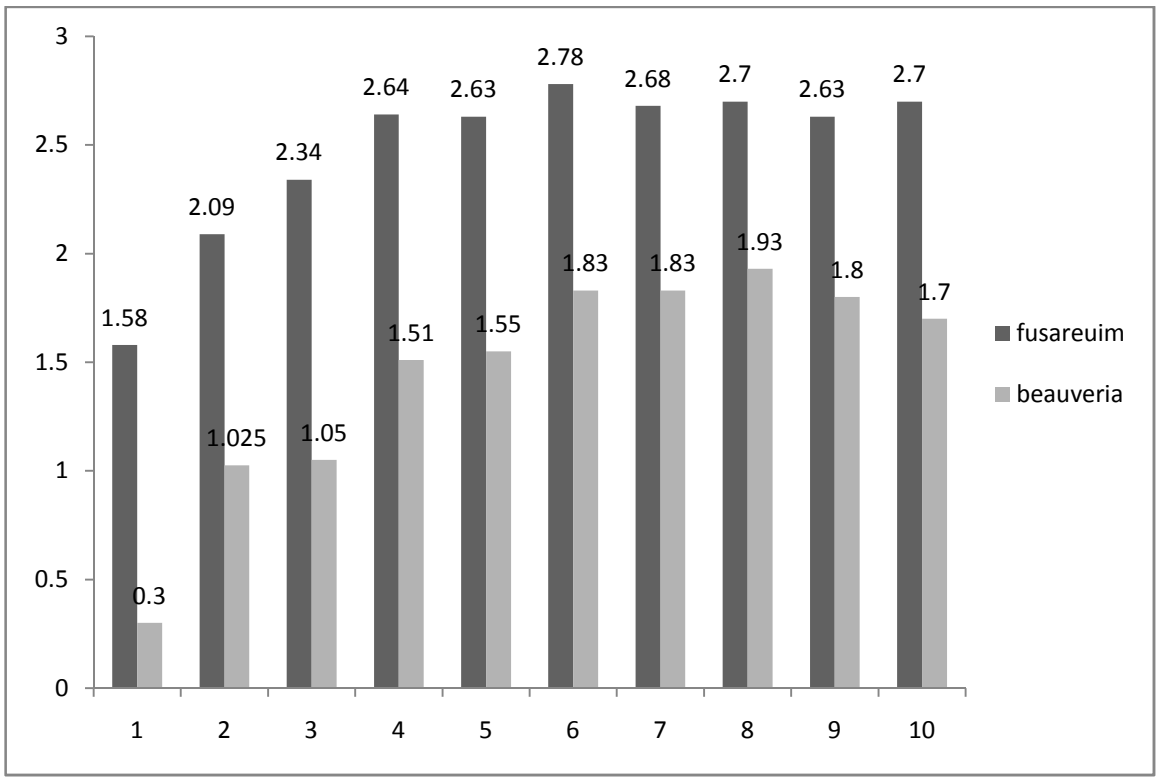

Figure 1. Effect of *Beauveria bassiane* on the growth of *Fusarium solani*

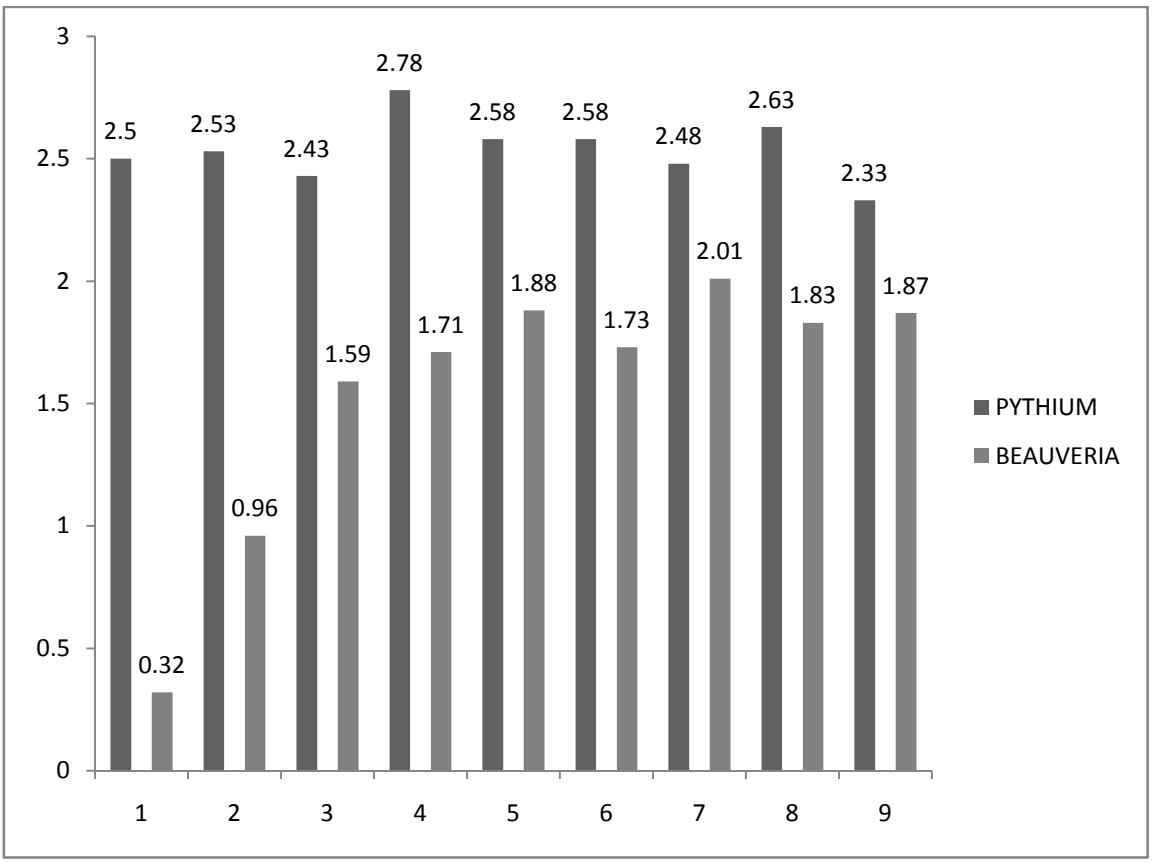

Figure 2. Effects of *Beauveria bassiane* on the linear growth of *Pythium digitatium in vitro*

4.2.4 The effects of coriander ethanol extracts and fungicide against *Pythium digitatium* after three days inoculation *in vitro*

The results in (Table 2) showed that the ethanol extracts of Coriandera and fungicide exhibited an inhibitory effect on the fungal growth after three days from inoculation. Among plant extracts coriander ethanol extracts at all concentration (12.5%, 25%, 50%, 100%) gave the highest inhibition of mycelia growth (.70,.73,.70 and.70) compared with the control.

4.2.5 The effects of coriander ethanol extracts and fungicide against *Pythium digitatium* after four days inoculation *in vitro*

The results in (Table 2) showed that the ethanol extracts of all plant tested and fungicide exhibited an inhibitory effect on the fungal growth after three days from inoculation. Among plant extracts coriander ethanol extracts at all concentration (12.5%, 25%, 50% and 100%) gave the highest inhibition of mycelia growth (.46,70,78, and.70) compared with the control.

4.2.6 The effects of coriander ethanol extracts and fungicide against *Pythium digitatium* after five days inoculation *in vitro*

Five days after incubation the results (table2) showed that the ethanol extracts of all plant tested and fungicide exhibited an inhibitory effect on the fungal growth after three days from inoculation. Among plant extracts coriander ethanol extracts at all concentration (12.5%, 25%, 50%, 100%) gave the highest inhibition of mycelia growth (.70, .70, .80 and .70) compared with the control.

4.2.7 The effects of *Beauveria bassiane* on potato plant height treated by *Fusarium solani* and *Pythium digitatium* after 20 days *in vivo*

The results in (Table 3) showed that the effect of *Beauveria bassiane* on potato treated by *Fusarium solani* and *Pythium digitatium* exhibited an tallest

plant height effect after 20 days from plant growth. (potato+ *Beauveria*, potato+ *Fusarium* + *Beauveria*, +potato+ *Pythium* +*Beauveria* +) gave (3.84, 3.71 and 3.89cm).compared with control (3.99cm).

4.2.8 The effects of *Beauveria bassiane* on potato plant height treated by *Fusarium solani* and *Pythium digitatium* after 30 days *in vivo*

The results in (Table 3) showed that the effect of *Beauveria bassiane* on potato treated by *Fusarium solani* and *Pythium digitatium* exhibited a tallest plant height effect on the field after 30 days from plant growth. (Potato+ *Beauveria, potato* +*Fusarium* + *Beauveria*,+ potato+ *Pythium* +*Beauveria* +) gave (3.84, 3.95, 4.32cm) compared with control (4.25cm).

4.2.9 The effects of *Beauveria bassiane* on potato plant height treated by *Fusarium solani* and *Pythium digitatium* after 40 days *in vivo*

The results in (table 3) showed that the effect of *Beauveria bassiane* on potato treated by *Fusarium solani* and *Pythium digitatium* exhibited an tallest plant height effect on the field after 40 days from plant growth.(potato+ Beauveria , potato +*Fusarium* + *Beauveria* ,+potato+ *Pythium* +*Beauveria*) gave (4.18, 4.14, 4.32cm) compared with control (4.47cm).

4.2.10 The effects *of Beauveria bassiane* on potato shoot (fresh weight) treated by *Fusarium solani* and *Pythium digitatium in vivo*

The results in (Table 4) showed that the effects of *Beauveria bassiane* on potato shoot (fresh weight) treated by *Fusarium solani* and *Pythium digitatium* exhibited an shoot fresh weight effect on the field.(potato+ *Beauveria* , potato +*Fusarium* + *Beauveria* ,+potato+ *Pythium* +*Beauveria*) gave (1.85, 2.87and 2.69g) compared with control (3.72g).

4.2.11The effects of *Beauveria bassiane* on potato shoot (dry weight) treated by *Fusarium solani* and *Pythium digitatium in vivo*

The results in (Table 4) showed that the effect of *Beauveria bassiane* on potato shoot(dry weight) treated by *Fusarium solani* and Pythium digitatium exhibited an inhibitory effect on the field.(potato+ *Beauveria* , potato +*Fusarium* + *Beauveria* ,+potato+ *Pythium* +*Beauveria*) gave (1.85, 1.29 and 1.21g) compared with control (1.48g).

4.2.12 The effects of *Beauveria bassiane* on potato Root (fresh weight) treated by *Fusarium solani* and *Pythium digitatium in vivo*

The results in (Table 5) showed that the effect of *Beauveria bassiane* on potato root (fresh weight) treated by *Fusarium solani* and *Pythium digitatium* exhibited a root (fresh weight) effect on the field. (potato+ *Beauveria*, potato +*Fusarium* + *Beauveria*, +potato+ *Pythium* +*Beauveria*) gave (2.78, 3.53 and 3.60g) compared with control.

4.2.13 The effects of *Beauveria bassiane* on potato Root (dry weight) treated by *Fusarium solani* and *Pythium digitatium*

The results in (Table 5) colum2 showed that the effect of *Beauveria bassiane* on potato root (dry weight) treated by *Fusarium solani* and *Pythium digitatium* exhibited an inhibitory root (dry weight) on the field (potato+ *Beauveria*, potato +*Fusarium* + *Beauveria* ,+potato+ *Pythium* +*Beauveria* +) gave (1.22, 1.62 and 1.68g) compared with control.

4.2.14 The effects of *Beauveria bassiane* on potato Yield treated by *Fusarium solani* and *Pythium digitatium*

The results in (Table6) showed that the effect of *Beauveria bassiane* on potato yield treted by *Fusarium solani* and *Pythium digitatium* exhibited an inhibitory effect on the field.(potato+ Beauveria , potato +Fusarium + Beauveria ,+potato+ pythium +Beauveria +) gave the highest yield on potato (2.25, 2.08 and 1.73) compared with control.

2.4.15 The effects of *Beauveria bassiane* on potato tuber weight treated by Fusarium solani and *pythium digitatium*

The results in (Table 7) showed that the effect of *Beauveria bassiane* on potato yield weight treated by *Fusarium solani* and *Pythium digitatium* exhibited an inhibitory effect on the field. (potato+ Beauveria , potato +Fusarium + Beauveria ,+potato+ pythium +Beauveria +) gave the highest tuber weight on potato growth (5.87, 5.75 and 6.64g) compared with control.

2.4.16 The effect of Beauveria bassiane in linear growth of *Fusarium solani in vitro.*

The growth of *Beauveria bassiane* was very slowly in culture media in PDA (Potato Dextrose Agar) compared with *Fusarium solani growth.* The growth of *Fusarium solani* covered all the plate in 7 days but in *Beauveria bassiane* the growth covered all the plate in 15 days, although the *Beauveria bassiane* is scattered the spores under and around the Fusarium growth.

Figure 1 showed that the effects of *Fusarium solani* after (3days, 5days, 7days and10 days) was gave (1.58, 2.34, 2.78 and 2.7 mm).The effects of *Beauveria bassiane* on *F.solani* treated after (3days, 5days, 7days and10 days) was gave (0.3, 1.05,1.83 and 1.93mm).

2.4.17 The effects of Beauveria bassiane in linear growth of *Pythium digitatium in vitro*

The growth of *Beauveria bassiane* was very slowly compared with *Pythium digitatium.* The growth of the fungus *Pythium digitatium* was faster than Fusarium solani it covered all the plate in 5 days but in *Beauveria bassiane* the plate was covered in 15 days, although the fungus *Beauveria bassiane* is very strong than *Pythium digitatium*

Figure 9 showed that the effect of *Pythium digitatium* after (3, 5, 7 and 10 days) was gave (2.5, 2.43, 2.58 and 2.33mm).The effect of *Beauveria bassiane* after (3, 5, 7 and 10 days) was gave (0.32, 1.59, 1.88 and 1.87mm).

CHAPTER FIVE

DISCUTION

Wilt and damping off are economically important diseases on potato in Sudan. The aim of this study was to solving the problem of wilt and damping off disease in Sudan. The estimation of yield losses by individual pests, Weed or disease ranged from 5 to 10% in temperate regions and from 50 to 100% in tropical regions (Van Emden 1988) .This means that the wilt and damping off of potato can cause considerable losses in tropical areas like the Sudan .Isolation of the fungus was carried out from wilted potatoes plants from Khartoum central market. The identification depended on laboratory investigations.

Fortunately, progress achieved in recognizing antimicrobial compounds in higher plant gave more promises in combating plant pathogenic disease. Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases (Schmutterer, 2002).

The antifungal effect of crud medicinal plant extracts Coriander was determined by *in vitro* study using ethanol as solvents .Four concentration of plant extracts were used (100, 50, 25, and 12.5) as antifungal activity against *Fusarium solani* and *Pythium digitatium. Plant-d*erived compounds (phytochemicals) have been attracting much interest as natural alternatives to synthetic compounds.

The present investigative revealed that *in vitro* growth of *Fusarium solani* and *Pythium digitatium* were significantly checked by ethanol extract of Coriandera at all concentration. Results showed that, Coriander had the highest antifungal activity against *Fusarium solani* and *Pythium digitatium* but inhibited was increase in low concentration 12.5 as it in habited 100% .All concentration tested significantly reduced the fungal growth compared with the control. Similarly result reported by (Abdel-Kader, *et al*. (2012) were

evaluated antifungal potential of various plant extracts against *Alternaria alternata*, *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *pythium sp.* and *Alternaria solani*. The results revealed that ginger extract had maximum inhibition on the growth of pathogenic fungi.

The inhibitory effect of the tested plant extracts may be due to their direct toxic effect on the pathogen as reported by (Vijayan, 1989). Investigations on the mechanisms of disease suppression by plant products have suggested that the active principles present in plant extracts may either act on the pathogen directly or induce systemic resistance in host plants resulting in a reduction of the disease development (Kagale *et al*., 2004).

The findings in this study confirmed that plant extract can be used as natural fungicides to control pathogenic fungi and thus reduce dependence on the synthetic fungicides. This study is only a preliminary one. More studies are still needed in the future to test the antifungal activities of the studied plant extracts on other pathogenic fungi.

In biological control *Beauveria bassiane* is known as entomofungal but it gave a good result in control *Fusarium solani* and *Pythium degitatium* although it have slow growth in culture media (potato dextrose agar) it cover the plate in 15 days but in soil media (sand and corn meal) have good and quake growth it cover the flask in 7 days because it is a soil born pathogen. It gave a pest result in vivo and quickly than the result in vitro. Generally it was scatter their spore around and under the growth of fungi and make strong colony like cotton *Fusarium solani* and *Pythium digitatium* cannot attack *Beauveria bassiane* finally it stopped the growth of two fungal.

Finally, this study is only a preliminary one. More studies are still needed in the future to test the antifungal activities of the Beauveria bassiane.

Conclusion

Ethanolic extract of the plant Coriander (*Coriandrum sativum*) ,*Beauveria bassiane* and fungicide Score against *Fusarium solani* and *Pythium degitatium* inhibited *in vitro.* Botanical extracts of Coriandera may offer a better alternative to synthetic fungicides.

CHAPTER SIX

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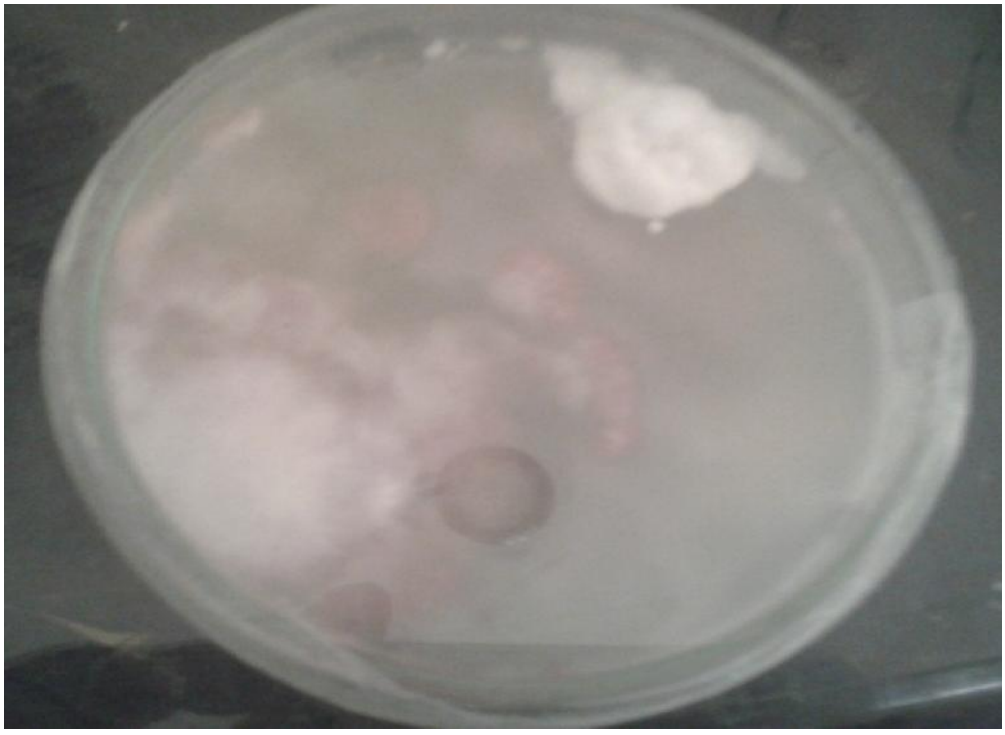
Appendices

Bu+Py

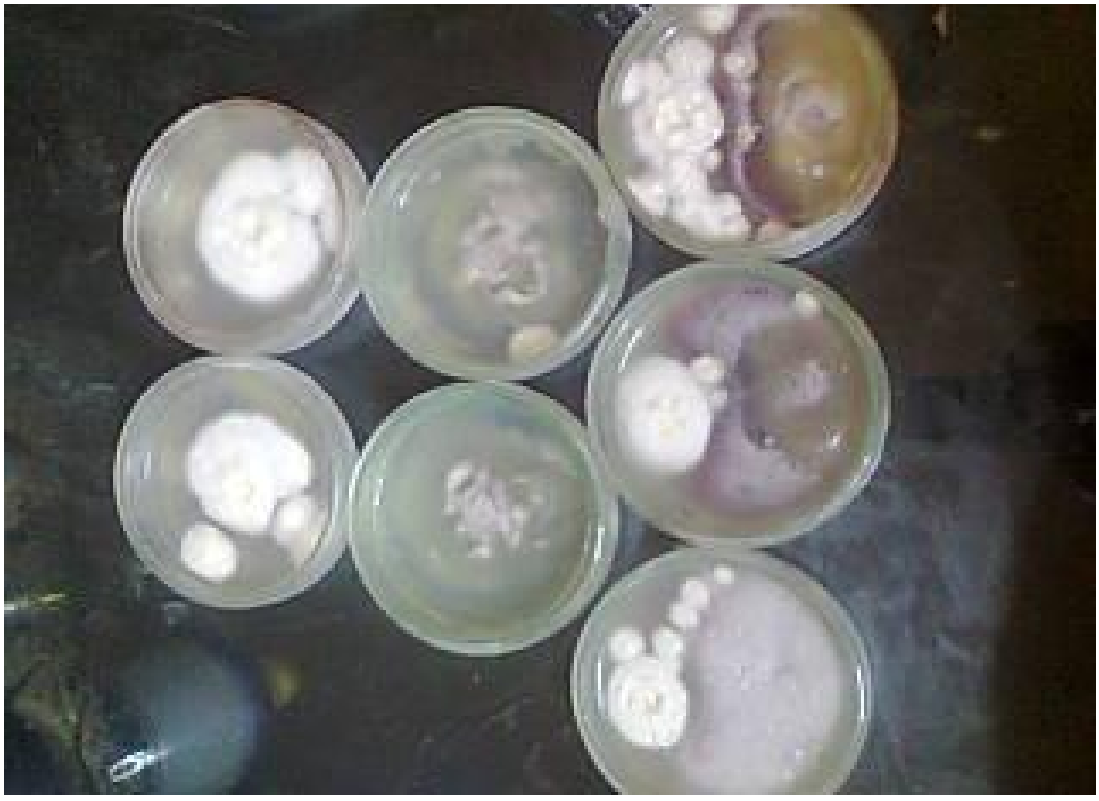
Bu+Py

Bu+Py



Fu+Bu

Fu+Bu

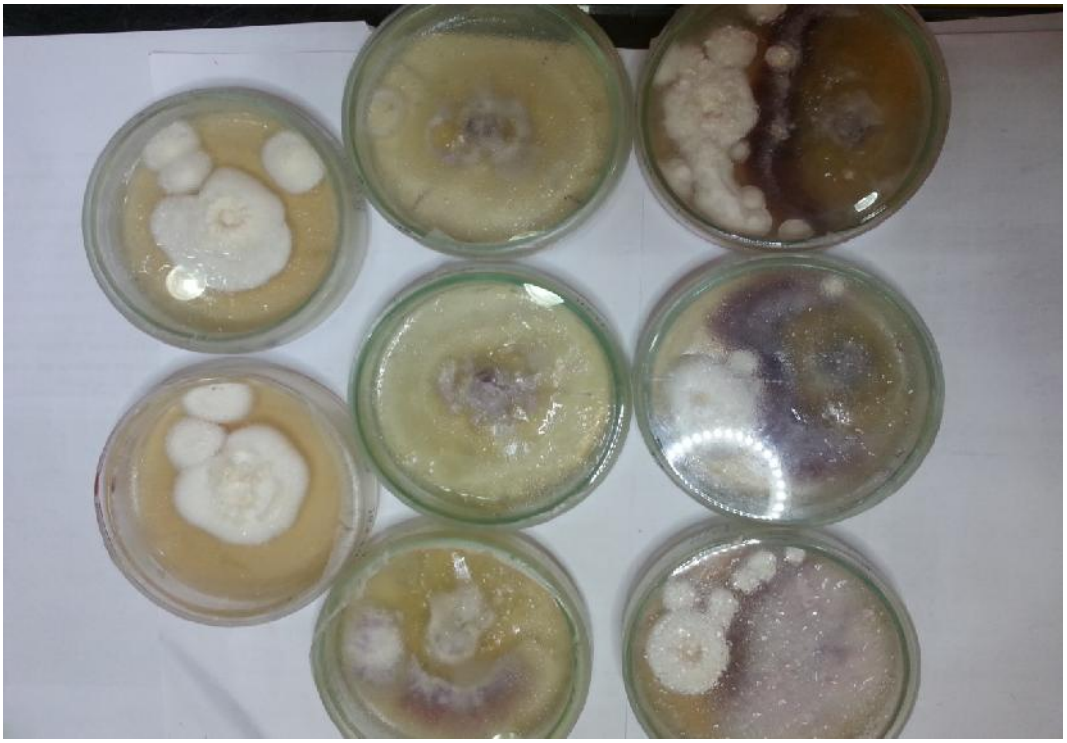


Fu+Bu

Bu+ Py

Pu+Fu

Pu+Fu



Pu+Fu