Deanship of Graduate Studies

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Bioactivity against plant fungal pathogens and germination studies of *Salvia fruticusa Mill* and *Organium syriacum*

Fatima Al.Zahra' Hussamaldein Manasra

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Prepared by:

Fatima Al.Zahra' Hussamaldein F.Manasra

B.Sc. plant production and protection , Hebron University, Palestine

Supervised by:

Dr. Khaled Sawalha

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Thesis Approval

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Prepared by: Fatima Al.Zahra' Hussamaldein Manasra

Registration No.: 21712753

Supervisor: Dr. KhaledSawalha

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The names and signatures of the examining committee members are as follows:

1-Head of Committee/ **Dr. Khaled Sawalha** **Signature:**.....

2- Internal Examiner/ Dr. Fuad Al-Rimawi **Signature:**.....

3- External Examiner/Dr. Raed Alkowni **Signature:** .....

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Dedication

To whom was the best help for me on this journey, to the permanent bond and shoulder , to who planted the seed of love of learning and knowledge in us, to my dear father Hussamaldin.

To the soul who was with me every step of my life to the spirit of my pure mother "Aminah"

To the one who fights the universe in our path, to who does not forget me in her praying to my dear mom," Suha".

To my husband Tamer, who has been a constant source of support and encouragement during the challenges of graduate college and life.

To my children, Jamil, Mariam and Mazen, you are my inspiration to achieve greatness, you have made me stronger, better and more fulfilled than I could have ever imagined. Without you, I would not be where I am today, I love you to the moon and back.

To those who are a piece of the heart, my brothers and sisters , Ala'a , Aminah ,Batool, Hammam , Abdulla, Seif and Mohamed who never stop giving themselves in countless ways

To childhood , young and age friends Daniyah who have always been a major source of support, you never fail to make me smile

To all my supportive friends during this journey

First and foremost, to a homeland that does not tire of containing us to our dear Palestine

Fatima Al.Zahra'

Declaration

I certify that the thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not be submitted for a higher degree to any other university or institution.

Signed: 

Fatima Al.Zahra' Manasra

Date: 3/6/2020

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Fatima Al.Zahra' Manasra

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Abstract:

Botrytis cinerea is the most common causative agent of a disease of gray molds which is one of the most destructive diseases infecting over 230 different plants worldwide such as tomatoes, grapes, and strawberries causes many economic losses. Also, *F. oxysporum* a soil-borne facultative pathogen with a global distribution, causes perennial wilt and foot-, root- and bulb disease in a wide range of economically important crops such as tomatoes sweet potatoes, legumes, cucurbits and bananas, both fungi can be controlled by several ways, such as cultural and chemical controls. Unfortunately, they develop high resistance to several chemical fungicides which have a negative environmental, plant, animal and human impacts. So several alternatives were suggested including biological control and plant extracts to reduce them. In recent decades medicinal plant including *S.fruticosa* and *O. syriacum* are being used as a potential source of significant compounds in medicines, cosmetics, and food industry. Because of the safety concerns it contains, and their bioactive essential oils which proved by several studies that have antifungal and antioxidant effects. Thus the objective of this study was to investigate the antifungal properties of Thyme and Sage natural products against *B. cinerea* and *F. oxysporum*. Sage and Thyme leaves were collected in 2019 from different locations in Bani Na'im. Then leaves extracted by using 99.0% ethanol, and essential oils were isolated by steam distillation. Through this process hydrosol obtained, the effect of each natural product against *B. cinerea* and *F. oxysporum* tested by using Mycelium growth rate assay . The media used for fungal is Potatoes Dextrose Agar (PDA). The results showed that both plants leaves extracts , Essential Oils and hydrosol had an antifungal effect on mycelium growth against both pathogenic fungi compared to the control sample (without adding any natural products) . The results showed that *S.fruticosa* leafs extracts , EOs and hydrosol

had an antifungal effect on mycelia growth of *B. cinerea* and *F. oxysporum* pathogens. Statistical analyses showed that there is significant differences in the rate of reduction (R) as the concentration of leaves extract or EOs increases until they totally inhibited (100%) both fungi growth . On the other hand, statistical analyses showed that there are no significant differences in the rate of reduction from hydrosol at the used concentrations. In addition to that *O. syriacum* results showed high antifungal activity of its leaves extract and essential oil that inhibit the mycelium growth rate (R) reached up to 100% against both pathogenic fungi and the minimum inhibition concentration of their leave extracts and EOs were (0.01%) . However, *O. syriacum* hydrosol wasn't significant in the the rate of reduction (R) of *B. cinerea* and *F. oxysporum* at the used concentrations .

The results of *S.fruticosa* and *O. syriacum* leaves extract , essential oils and hydrosol antifungal activity and their antifungal agents are of particular importance for managing pathogenic fungi ,so it’s very important to increase .The cultivated areas of sage and thyme ,in order to efficiently use them to produce bio- pesticides. In order to improve Sage and Thyme germination , many different treatments were done through this experiment, in order to study their effect on seed germination , for achieving rapid, uniform and higher germination which will lead to higher plant stand establishment. In order to increase seed germination due to their economic value . six treatments and control which were subjected to sand paper scarification , chilling, growth regulator treatment using Gibberellins and kinetin in 500 ppm for 24hours and 48 hours were done . After each treatment, the seeds were cultivated within the planting bed and placed on filter paper. Germination counting was started after 3days. Results showed the effect of different treatment on sage seed germination rate . Kinetin growth regulator treatment for 24 hours showed the highest germination percentage (80.7%) compared to the control one. However chilling showed the lowest germination percentage(22.5%) among all treatments. On the other hand, result

of the treatment on thyme showed that, the highest germination percentage with a numeric value of 91.4% was observed in the kinetin growth regulator for 24 hours , which compared to the control treatment was increased by 45.8%. Next higher percentages of germination were obtained for sand paper scarification with a numerical value of 74.7% and gibberellic acid treatment for 24 hours treatment with the value of 55.7% and the lowest was growth regulator kinetin treatment for 48 hours with percentage of germination was (17%) .From all results of this study, *S.fruticosa* and *O. syriacum* leaves extracts , essential oils and hydrosol antifungal activity against *B. cinerea* and *F. oxysporum* , can be used as a fungicide to reduce disease caused by fungi, and recommended to formulate fungicide with adding *S.fruticosa* and *O. syriacum* natural products .

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LIST OF ABBREVIATIONS

MGR	Mycelium Growth Rate
PDA	Potatoes Dextrose Agar
GR	Germination rate
S .fruticosa	*Salviafruticosa*
O. syriacum	*Origanum syriacum*
B. cinerea	*Botrytis cinerea*
F. oxysporum	*Fusarium oxysporum*
BCA	Biological Content Agency
EOs	Essential Oils
MIC	Minimum inhibition concentration
PY	Physical dormancy
MPD	Morphophysiological dormancy
MD	Morphological dormancy
PD	Physiological dormancy

Chapter One: Introduction

1.1 Botanical background:

1.1.1: Lamiaceae family

Medicinal plants include various species of plants ranging from wild to crops. Such plants are used in herbalism and there are medicinal practices in some of these plants. Medicinal plants are known to be rich sources of bioactive ingredients that can be used to produce and synthesize drugs (Rasool,2012). Such plants also play an important role in the development of human cultures (Ogunjobi and Abiola, 2013).

Most medicinal plants have been studied to provide biologically active compounds based on most of our current drugs. There's much more to discover about their precise pharmacology though. This is particularly relevant as regards essential oils that have such a condensed yet complicated composition. But as written by Lawless (1992) In the Encyclopedia of essential oils that ' only a small proportion of the world's floral compounds have been tested for pharmically active compounds, but with the danger of extinction of plants, there is a real risk of the loss of many important plant sources. "(Lawless, 1992)

formerly called *labiateae* family of mint is an important family of medicinal and aromatic plants. It includes about 236 genera and over 6,000 species, with *Salvia, Scutellaria, Stachys, Plectranthus, Hyptis, Teucrium, Vitex, Thymus, and Nepeta* being the main genera. It is with a cosmopolitan distribution with great diversity and variety . the species share various natural environments and many of ecosystems.(Lawrence.1992) Although some species are woody shrubs or subshrubs, most family members are perennial or annual herbs with square stems. Typically the leaves are simple and contrary to the arrangement; most are fragrant and contain volatile oils. The flowers are typically arranged in clusters and have tubular corollas (united petals) with five lobes of bell-like calyxes (united sepals)

that are two-lipped, open-mouthed. Generally the fruit is a dry nut. It is distributed widely in the world's tropical and subtropical regions. Its contains more than 450 species. The plants belonging to the *Lamiaceae* family are well known for having essential bio-active properties These plants were used in various indigenous medicine systems.(Inc.2019)

Table1.1: Classification of two species for Sage & Thyme.).(Danin & Fragman.2016).

Rank	Sage	Thyme
Kingdom	Plantae – Plants	Plantae – Plants
Subkingdom	*Tracheobionta* – Vascular plants	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants	*Magnoliophyta* – Flowering plants
Class	*Magnoliopsida* – Dicotyledons	*Magnoliopsida* – Dicotyledons
Subclass	Asteridae	Asteridae
Order	Lamiales	Lamiales
Family	*Lamiaceae* – Mint family	*Lamiaceae* – Mint family
Genus	*Salvia L.* – sage P	Origanum
Species	*Salviafruticosa Mill.*	*Origanum syriacum*

1.1.1.1 *Salvia fruticusa Mill* ,Sage:

1.1.1.1.1 Botanical Description:

Salvia is an evergreen shrub of the Labiatae family. This plant grows in height of 30-70 cm. The stem, with many leaves, is woody, tetragonal at the base. The leaves are opposite, small, glandular, rough, finely dented, the lower unusual, the higher sessile, whitish underneath and grayish-green above as(figure 1.1) shows. The flowers are purple-blue, up to 3 cm long, with bilabiate corollas clustered in terminal spikes, in groups of 7-10, deeply aromatic, with rich nectar; they develop from early summer until early autumn. *S. fruticusa* usually grows up to 750 m above sea level on dry hillsides, on clay soils, in sunny places. The leaves of numerous plants, are densely covered with glandular and non-glandular trichomes, which originate from epidermal cells (Werker, 2000). Developmental and structural studies of trichomes can shed light on the nature of the secreted material and their functional significance. Plant species bearing glandular trichomes generally produce relatively large amounts of bioactive compounds.(Duke, 1994)

Figure 1.1: *S. fruticusa,* Sage plant.

Table 1.2 **:** Botanical Description for Sage (*S. fruticusa*).(Danin & Fragman.2016).

Plant structure		**Flowering and reproduction**	
Life form (Raunkiaer)	chamaephyte	Petal or tepal color	lilach
spinescence	absent	Sexuality and reproductive morphology	Flowers hermaphrodite only
succulence	Non-succulent	Sporangia or seed homogeneity	Homogenous seed-fruits
Summer shedding	Perennating	Flowering time	August, October April, July, June, May, March, November, September
Leaf arrangement		**Habitat**	
Leaf arrangement	Opposite (2 leaves / nod)	Habitat	Mediterranean maquis and forest
Leaf type	dissected once, entire.	chorotype	Mediterranean
Leaf or leaflet margin	Smooth	Salt resistance	glycophyte
stipule	absent	synanthorop	Obligate natural

1.1.1.1.2 Geographical distribution:

Salviafruticosa Mill . (Labiatae) is an east Mediterranean basin native species distributed from Italy, Sicily and Cyrenaica via the south Balkan Peninsula (Albania and Greece) to west Syria. (Hedge, 1982)

Table 1.3: Geographical distribution for Sage (*Salvia fruticusa*).(Danin & Fragman.2016).

Upper Galilee	**Common**
Lower Galilee	(Common)
Mt. Carmel	Very Common
Coastal Galilee	Very Common
Acco Plain	Common
Coast of Carmel	Very Common
West bank	Very Common

1.1.1.1.3 Economic importance:

It is one of the most economically important species of Salvia, valued for its beauty, medicinal properties, culinary use along with its sweet nectar and pollen, and in Greece it has had a particularly long history of its use. The leaves of sage are used as a spice or adulterant. The leaves comprise 50-95% of the sage leaves that are commercially dried. A aromatic tea is made by infusing the leaves. *S.fruticosa* has been studied from various aspects, such as chemical composition or genetic variation of essential oils , antimicrobial, cytotoxic, antiviral, antifungal, antioxidant effects of essential oils and extracts (Bayrak et Augul, 1987)(Müller, *et al*., 1997)

The leaves are antispasmodic, antihydrotic, antiseptic, astringent, carminative, cholagogue, depurative, expectorant, febrifuge, stimulant, tonic, and vasodilator. These are used

internally to treat gastrointestinal and respiratory problems, menstrual disorders, miscarriage, nervous stress and depression. The leaves can be harvested as required and used fresh, or they can be harvested before the flowers open and dried or distilled for their essential oil. (Ken Fern.2016)

1.1.1.*2 Origanum syriacum* L , Thyme:

1.1.1.2.1 Botanical Description:

O. syriacum Perennial weed, 60 cm-90 cm tall, with prolific woody roots, branched woody, hairy stems. The leaves are opposite, small or sub-sessile (petitolate to 8 mm), ovate, 5 mm–35 mm roughly 4 mm–23 mm and hairy, the margins are complete or distant, the apex is obtuse as (figure 1.2) shows. The upper surface of the leaf is darker; the lower surface of the leaf is shinier with secretory glands. (Alma,2003) Flowers are hairy and shortly petiolate. Bracts obviate or elliptic, 2 mm – 5 mm, 1.5 mm – 3.5 mm, full or denticulate, acute or obtuse. A two-lipped pale violet corolla 4.5 mm-7.5 mm and a calyx of five-toothed campanulas.(Kintzios,2004)

Figure 1.2: *Origanum syriacum L* , Thyme plant.

Table 1.4**:** Botanical Description for *Origanum syriacum L*, Thyme.

(Danin&Fragman.2016).

Plant structure		**Flowering and reproduction**	
Life form (Raunkiaer)	chamaephyte	Petal or tepal color	lilach
spinescence	absent	Sexuality and reproductive morphology	Flowers hermaphrodite only
succulence	Non-succulent	Sporangia or seed homogeneity	Homogenous seed-fruits
Summer shedding	Perennating	Flowering time	August, October April, July, June, May, March, November, September
Leaf arrangement		**Habitat**	
Leaf arrangement	Opposite (2 leaves / nod)	Habitat	Mediterranean maquis and forest
Leaf type	dissected once, entire.	chorotype	Mediterranean
Leaf or leaflet margin	Smooth	Salt resistance	glycophyte
stipule	absent	synanthorop	Obligate natural

1.1.1.2.2 Geographical distribution:

Grew up in a large area of the eastern Mediterranean. It can be found in southern Turkey, on Cyprus, in Syria, Lebanon, Palestine, Jordan, and on the Sinai Peninsula and develops of almost sea level to at least 2000 m in rocky soils, often on calcareous Mountainous Southern Sinai (location: St. Katherine-Specific Location: Wadi shagg musa), Galala Desert, Isthmic Desert. It inhabits large area in the eastern Mediterranean .In South Lebanon, *O. syriacum* grows naturally in mountains and regions that surround the Litanie River(Aslim and Yucel, 2008)

1.1.1.2.3 Economic importance:

The leaves and flowering tops are used as a seasoning with a taste that is reminiscent of a mixture of thyme, marjoram and oregano. Sometimes the dried herb is combined with sumac (from the species of Rhus) to form the spice mixture known as ' zatar, ' which is used as a topping for bread along with olive oil. The Bedouin grind the dried leaves, add salt and eat the dry mixture on bread.(Facciola,1990) The leaves and flowering stems of this species are often dried and supplied commercially as 'oregano'.(Bown,1995) . It has antiseptic properties, and is able to relieve pain in the stomach and intestines. It is also used to treat problems with heart, cough, toothache, cold, anxiety and wounds(Gardner et al., 1989).
O. syriacum Also have antimutagenic, antitumor and cytotoxic, antioxidant, anti-inflammatory, antispasmodic, expectorant, carminative and antitussive properties. In addition, Origanum can be used to treat vomiting and rheumatism, asthma, hemorrhoids, sexual diseases, bites of animals and poisoning, as well as diabetes and obesity management. This plants were also used as carminatives, diaphoretics and tonics, and maybe as a source of antimicrobials. (García,2016).Because of their essential oils '

antioxidant, antimicrobial, insecticidal and antifungal function, Origanum species have recently been of great interest as potential medicinal substances and as natural additives to substitute synthetic products in the food industry(Ocana-Fuentus et al., 2010).

1.2 Fungal background:

1.2.1 *Botrytis cinerea*

1.2.1.1 The host rang and importance:

Botrytis cinerea Pers ascomycete. is a necrotrophic plant pathogen and the causative agent of the disease commonly known as ' gray mold, ' resulting in major pre- and post-harvest losses of over 200 crop species worldwide.(Staats ,*et.al*.2005). It can cause soft rotting of all parts of the aerial plant and rotting of post-harvest vegetables, fruits and flowers to produce abundant gray conidiophores and (macro)conidia typical of the diseases shown in (figure 1.3A,BandC) But it usually gains entry into such tissues much earlier in crop development and remains quiet for a considerable amount of time before rapidly rotting tissues when the environment is suitable and the physiology of the host changes. Therefore, after harvesting apparently healthy crops and subsequent transportation to distant markets where the losses become apparent, serious damage is caused. however *B. cinerea* also causes massive losses before harvesting in some field- and greenhouse-grown horticultural crops, or even in some hosts at the seedling stage. Some monocotyledonous hosts can also be attacked by *B. cinerea* .(Staats *et. al*., 2005, 2007). Infections with *B. cinerea* causing post-harvest decline usually occurs on the field stage and may remain latent until is stored *B. cinerea* can grow from rotted fruit next to healthy fruit, causing extensive deterioration of the product and spoiling whole lots at times.

The asexual period of *B. cinerea* is known in the genus Botrytis, that belongs to the Moniliaceae family. All pathogenic Botrytis species are necrotrophic, as plant cells during pathogenesis are actively killed (Prins, *et. al*. .2000).

This fungus causes huge economic losses in dicotylledonic crops such as flowers (rose and gerbera flowers), fruits and legumes (grapes, strawberries, tomatoes, French beans, chickpeas) and oil plants (sunflower) .(Williamson, *et al* .2007).The fungus uses a broad range of infection techniques to directly penetrate mature-to-senescent leaves and other soft tissues, such as seedlings, floral organs, and mature fruits. In cool, humid weather (10 to 25 ° C) the fungus usually infects host tissues in water droplets (Prins *et al* .2000)

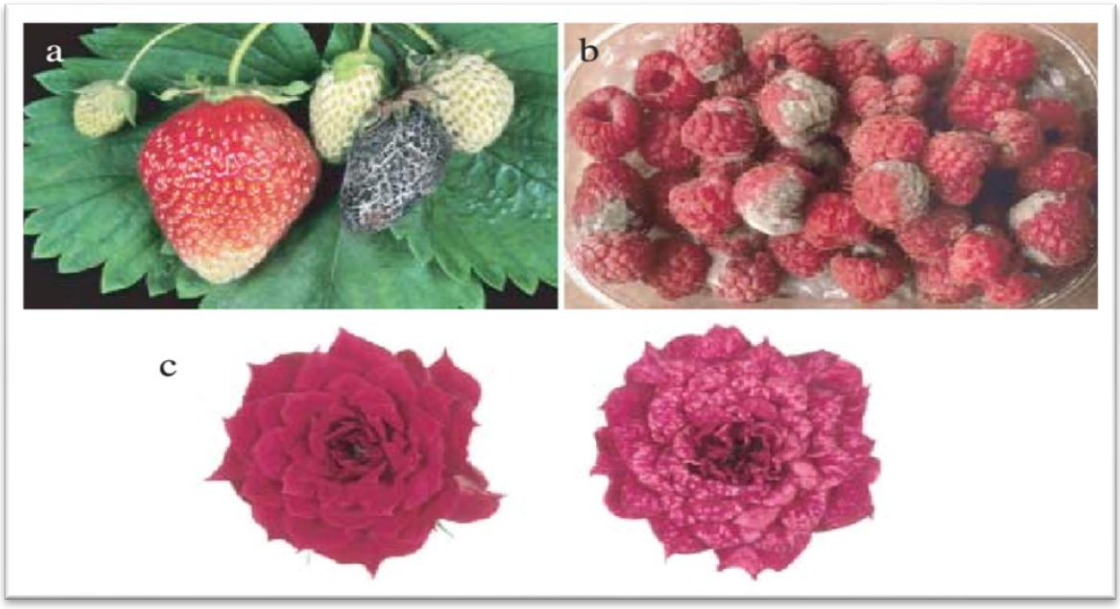


Figure 1.3: Symptoms of infection by *Botrytis cinerea*. (a) Grey mould of strawberry fruit. (b) Grey mould of raspberry fruit. (c) Rose petals(Williamson, *et al*.2007).

1.2.1.2. *Botrytis cinerea* lifecycle

In dying host tissues, sclerotia grows and constitutes an essential mechanism of survival in *B. cinerea*, but very variable in scale, is not easily visible in all susceptible crops. The

melanized rind and β-glucans that enclose the inner mycelium shield sclerotia from desiccation, UV radiation and long-term microbial attack (Backhouse and Willets, 1984.

The life cycle of *Botrytis* fungi begins with the release of vast amounts of asexual spores (conidia) from the tips of fungi's conidiophores. The spores continue to germinate when they land on the surface of the host plant. This also relies, among others factors such as temperature, humidity and sunlight. *Botrytis* species develop sclerotia during the winter season, which are melanized resting bodies. Compared to normal conidia formed in spring or warmer conditions, sclerotia is hardened more (due to the presence of melanin) allowing the spore to withstand harsh environmental conditions (overwintering).When the spores land on the surface of the host plant, they begin to germinate; within a few hours, they produce germ tubes. The development of structures such as appressorium (specialized flattened hyphae from which the penetration peg develops) follows this. The penetration peg and other hyphae continue to grow into the epidermal cells following penetration, thus releasing a variety of chemicals and metabolites that cause serious damage to the host plant cells. After the plant cells death, it has been shown that fungi such as *B. cinerea* live as saprophytes (or nectotrophs) where they continue to live and feed on dead plant cells. Conidiophores produced in the process release spores that can land on other host plants or on soil and repeat the cycle of life.(Bahar, *et .al*.2010)

Microconidia, which exists in all species of Botrytis, provides an alternative microscopic propagation for these fungi under adverse conditions. These are generally found in aged fungal cultures or those infected with other species and in combination with sclerotia. Microconidia grows from macroconidia-generated germ tubes, more mature hyphae, within empty hyphal cells, and from appressoria and sclerotia (Jarvis, 1980).

Most isolates produce abundant multinucleate (3-6 nuclei) conidia (macroconidia)which are unicellular, hyaline to slightly colored, smooth, ovoid to ellipsoid, and measure 10-12 ×8-10 µm. Conidia are produced on short sterigmata on the swollen tips of aerial, free, branched conidiophores (Holz *et al*., 2004)(figure 1.4 A,B).*B. cinerea* produces macroscopic, stipitate apothecia that originate from sclerotia (Faretra and Antonacci, 1987) (Figure1.4C).

The survival of *Botrytis* species ' mycelium under natural conditions has hardly been studied and it is often difficult to determine whether mycelium survival is involved or whether it includes microsclerotia or chlamydospores. There is some evidence that the mycelium of certain species of Botrytis, and particularly those more specialized in their parasitism, can survive in bulbs seeds and other vegetative plant parts for significant periods (Coley-Smith, 1980).

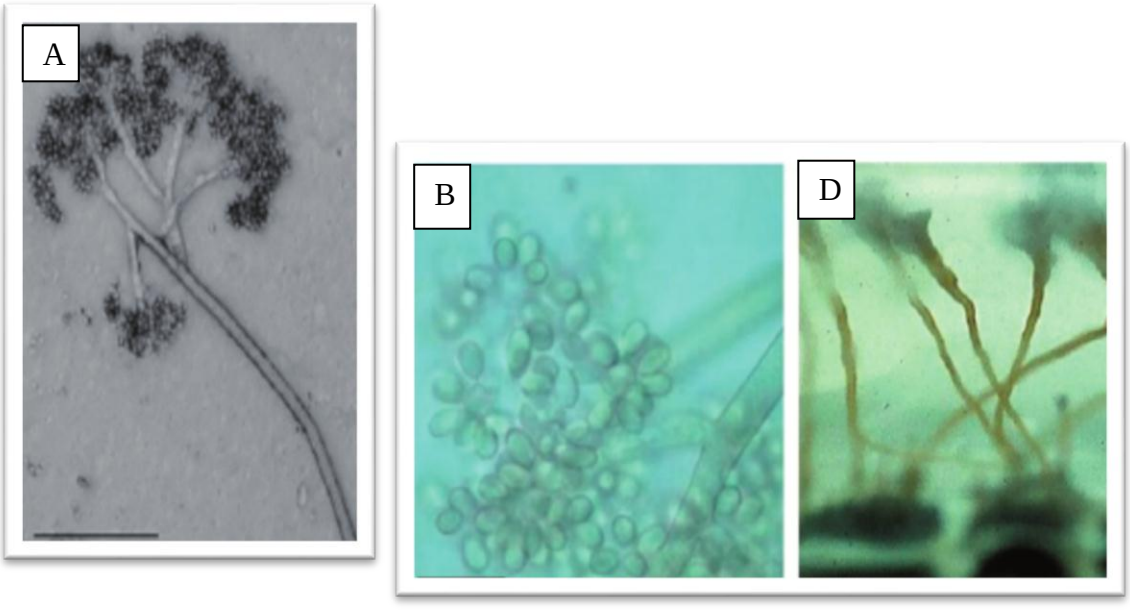


Figure 1.4: *Botrytis cinerea*. (A) Conidiophore, bar = 100µm. (B) Conidia, bar = 10 µm. (C) Apothecia.((Holz *et al*., 2004) (Faretra and Antonacci, 1987)

1.2.1.3. *Botrytis cinerea* Management:

B. cinerea is difficult to control because it has a range of attack modes, various hosts as sources of inoculum, and can survive as mycelia and/or conidia, or as sclerotia in crop debris for extended periods. For these reasons, it is doubtful that any single control intervention will be effective and a more detailed understanding of the host-pathogen relationship, the microenvironment in which the fungus occurs.(Brein. *et al*.2007).

1.2.1.3.1 Chemical control:

B. cinerea is among the first targets ever identified for chemical disease control. Nicot et al., 2000, stated that the Romans already used 11 elemental sulfur for the control of gray mold and mildew diseases in grapes. Fungicides are the primary method for the chemical treatment of gray molds (Nicot, *et al.*, 2000).

Several chemical classes of novel botryticides, with *B.cinereas* specificity Since the 1950s, cinereas have been developed with the advent of modern fungicides, such as aromatic hydrocarbons and dithiocarbons and dithiocarbamates. (Hayashi, 2003).

Chemical fungicides either destroy the fungus itself (fungicidal products) or prevent its development (fungistatics products). In both cases, the fungicide targets the fungus ' biological structure (e.g., cell wall), or biological activity (e.g. protein synthesis). Over time there is periodic natural selection, with resistant strains of the fungus surviving and eventually replacing the strains that are susceptible to the fungicide. The fungicides are slowly becoming less successful and must eventually either be changed or substituted by a completely new fungicide (Nicot ,*et al*.,2000).

Five types of fungicides are known, namely those that affect respiration ,microtubular formation, osmoregulation, inhibitors of sterol biosynthesis and those whose toxicity is reversed by amino acids (Rosslenbroich and Stuebler, 2000). Many multisite toxicants have been used agains *B. cinerea* , impacting the fungal respiration. Long-term without significant resistance in field populations (e.g., thiram, mancozeb, captan, dichlofluanide, tolylfluanide); (Pollastro, *et al*., 1996)

Strobilurin fungicides inhibiting B-control cytochrome *B. cinerea* and have the benefit of being broad-spectrum fungicides that could control several diseases. The anilinopyrimidines are useful botryticides that methionine and some other amino acids are antagonistic. These fungicides can prevent the secretion of hydrolytic enzymes, such as cutinases, lipas, which play a role in pathogenesis (Miura,*et al.,* 1994). Historically, chemical fungicides have proved to be non-specific, and can therefore act on species other than the target fungus, including other beneficial agents that occur naturally. They may also be harmful and non-biodegradable, because of their chemical nature. Chemical residues can build up all over the food chain and in the soil. Consumers around the world are becoming increasingly aware of the potential environmental and health problems associated with building-up toxic chemicals, particularly in food products. This has resulted in an increasing pressure from the user to reduce the use of chemical pesticides. "Organic" goods or the As a result of sustainable development programs-produced without the assistance of chemicals-they are increasingly seen as safer, more attractive and of premium value (Built and Dubos, 1988).

1.2.1.3.2 Biological control :

Biological control is based on the use of competitive or parasitic micro-organisms that may compete for space or nutrients may produce antagonistic antibiotics or hyper parasitize mycelium (Jakab, *et al.*,2001).

Early studies of the phyllosphere's microbial ecology showed that there was significant potential for the use of microbial antagonists to control Botrytis on crops. It has now approved at least seven products (Elad and Stewart, 2004**)** For use in greenhouses, under plastic tunnels or on the field in various countries for food and non-food plants. They reached niche markets in situations where heavy use of traditional fungicides was limited due to accumulated residues, or due to restrictions imposed by importing countries. The original expectations to infrequently deliver a single biological control agency (BCA) and then rely on its ability to self-disperse to the necessary target zones in a crop canopy have turned out to be unrealistically optimistic in most cases, but great advances have been made in understanding their biological modes of action. BCA formulations might include filamentous fungi such as Trichoderma harzianum, Clonostachys rosea, and Ulocladium oudemansii, Candida oleophila yeast, or bacteria such as Streptomyces griseoviridis, Bacillus subtilis, and Pseudomonas syringae. Many BCAs are sprayed on the crop field, but strawberries using bees with inoculation trays in the hives have had some success (Peng, *et al*., 1992).

The activation of mediated resistance includes different signaling pathways, such as systemically acquired resistance or induced systemic resistance. These pathways depend on signaling molecules like salicylic acid, ethylene, and jasmonic acid to varying degrees. Induction of systemic resistance may also be triggered by the use of salicylic acid or its counterpart, benzothiadiazole and ß- aminobutyric acid (Jakab *et al.*, 2001).

1.2.1.3.3 Cultural control

High humidity, low light and a mild temperature worsen the gray mold. It is therefore important to create an open canopy in crop management to provide adequate air movement and good light detection, so that water droplets from rain or irrigation dry as soon as possible. High RH helps conidial production and enables host germination and penetration Diverse and often unique to specific species and crop systems are cultural practices which mitigate the effects of gray mold. In perennial woody plants, such as grapevines, pruning has been proven helpful in reducing the plant's excessive vegetative growth (Gubler et al., 1987).

The key techniques for minimizing gray mold by cultural methods under greenhouse and field conditions are, according to Hayashi (2003),: i. Moisture management by drainage, water supply reduction and temperature control; ii. Inoculum decrease by removal of dead, decayed, or infected material; iii. Reduction of bird, insect fungal infections, hail and frost injuries; iv. Reduce crop density to create unfavorable microclimate for *B. cinerea*; v., nutrient conditions reduced, and vi. Use of greenhouse UV films to prevent conidia formation from activating. Consequently, cultural practices in many crops cannot provide sufficient control of the disease.(Hayashi, 2003)

1.2.2. *Fusarium oxysporum f. sp. Lycopersici*:

1.2.2.1. The host rang and importance:

F. oxysporum, a soil-borne facultative pathogen with a global distribution, causes perennial wilt and foot-, root- and bulb disease in a wide range of economically important crops (Beckman.1987). The genus Fusarium is one of the most complex and adaptive organisms in the Eumycota and the group of species *F. oxysporum* contains plant, animal and human pathogens and a diverse range of non-pathogens (Gordon, 2017). Fusarium species are

omnipresent soil-borne pathogens of a wide variety of horticultural and food crops that cause devastating vascular wilts, rots and damping -off diseases. (Bodah, 2017) *F. oxysporum* isolates are highly host-specific and have been grouped into special formations according to their host range (Armstrong and armstrong, 1981).There are three recognized physiological races within *F. oxysporum* (race 1, race 2 and race 3) that are differentiated between them based on their pathogenicity among various tomato cultivars consisting of monogenic dominant genes and race-specific. These genes of resistance to F. *oxysporum* found in wild tomatoes were introduced into commercial varieties (Biju et al., 2017). F. *oxysporum* strains are either saprophytic or non-pathogenic (Kumar et al., 2010). Phytopathogenic strains, however, cause destructive vascular wilt disease and often limit economically important crop production (Servin et al., 2015). *F. oxysporum* causes wilt disease in more than 150 hosts and range with specific formae specials (Bertoldo et al., 2015).

The most susceptible plants are tomatoes and other solanaceous crops, sweet potatoes, legumes, cucurbits and bananas, although they will also infect other herbaceous plants, cotton, ornamentals and palms. (Miller et al., 2011) as shown in (figure 1.5 A&B).

F. oxysporum caused major Tomato wilt diseases (Borisade ,*et al*., 2017). It's reaches the root epidermis, then spreads through the vascular tissue and inhabits the xylem vessels of the plant, causing the channel to become blocked, resulting in severe water stress as a result. (Singh, *et al*., 2017). It's progression of plant vascular infection is a complex phenomenon, and the sequential steps involved in the infection cycle are: (1) root identification by host-pathogen signals, (2) root hair surface attachment, and hyphal propagation, (3) invasion and differentiation of the root cortex and vascular tissue within xylem vessels, (4) finally oozing toxins and virulence factors. Vessel colonization

contributes to the development of disease and the characteristic wilting of the host plant (Di, *et al.*, 2016).

The characteristic wilt symptoms appear as a result of vessel blockage triggered by fungal hyphae collection and a combination of host-pathogen interaction such as the release of toxins, gums, gels, and tylosis formation. Typical indications of disease, such as epinasty of the leaves, clearing of the veins, wilting and defoliation, appear and eventually precede death of host plants. During this process, the vascular wilt fungus, which remains confined to the xylem vessels, propagates through parenchymatic tissue and starts to sporulate abundantly on the plant's surface, such as, leaf, steam etc. The spread of the pathogens will take place through seeds, transplants, soil or other means (McGovern, 2015; Renu Joshi, 2018).

Figure 1.5:Symptoms of *F . oxysporum* on tomatoes (A)Yellowing and death of leaves on one side of the stem, (B) Dark brown vascular discoloration. (Miller, *et.al*, 2003)

1.2.2.2. *F. oxysporum* lifecycle:

Fusarium species life cycle could be divided into dormant, parasitic, and saprophytic stages (Beckman, 1987). The dormant stage includes the inhibition and germination of soil-resting structures. The parasite stage consists of root penetration, colonization of the root cortex and endodermis, movement to the xylem, colonization of the xylem of stems and leaves, presentation of symptoms and, finally, death of the host .The saprophytic stage is the development of dead-host resting structures (Schnathorst, 1981). *F. oxysporum* is diversified by macroconid shape, microconidiophore structure, chlamydospore formation (Beckman 1987) ,as shown in(figure 1.5A,B&C) .

In adverse conditions the fungus causes sclerosis (singular= sclerotium). A sclerotium is the organized mass of hyphae that remains dormant under unfavorable conditions and germinates when favorable conditions return and become a source of infection. (Mui-Yun, 2003). Macroconidia and microconidia are usually formed from slender phialides by the fusarium species. Macroconidia is hyaline, fusiform to sickle-shaped, two to several cells, mostly with elongated apical cells and basal pedicellate cells .Microconidia is a 1 to2-celled fusiform, hyaline, pyriform, ovoid, straight or curved (Nelson, *et al.,* 1994)

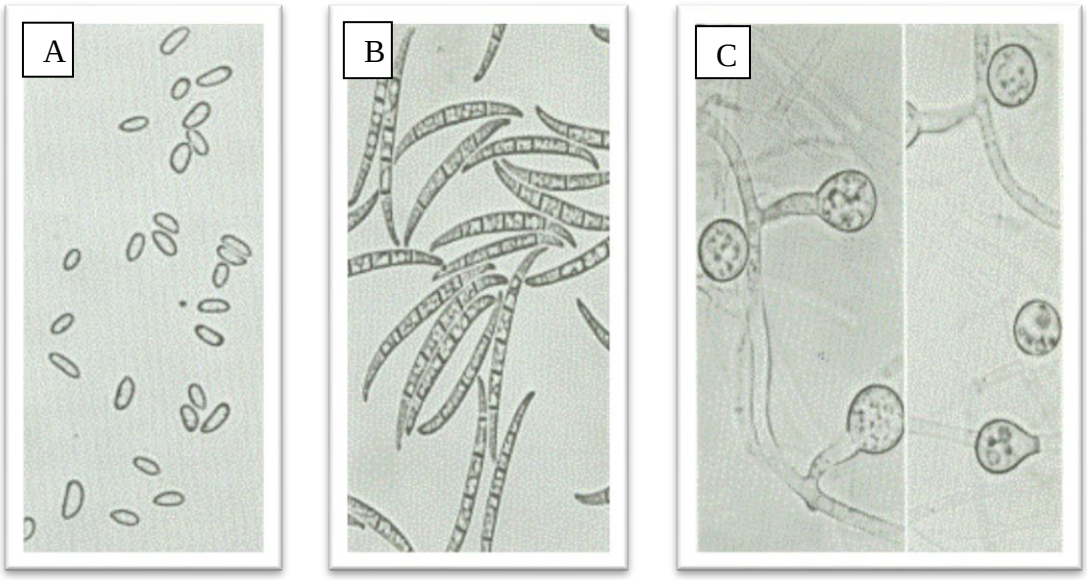


Figure 1.6: *Fusarium oxysporum* f. sp. *Lycopersici*(A), Microconidia; (B) Macroconidia;(C) Chlamydospores .(Toussoun, & Nelson. 1976).

1.2.2.3. *F. oxysporum* Management:

1.2.2.3.1.Chemical control :

Many chemical fungicides such as bavistin (Alam et al., 2010) , prochloraz and carbendazim (Song et al., 2004)and salicylic acid used to promote resistance to the disease, but these chemicals have a detrimental impact on human health and are environmentally harmful. (Amel et al., 2010).

1.2.2.3.2. Biological control :

The soil microbes such as *Trichoderma harzianum, T. asperellum, T. koningii, Penicillium* spp. And *Streptomyces griseoviridis* resides in the plant rhizosphere and has the ability to suppress the pathogens and stimulate plant growth through phytohormone synthesis and/or

degradation of complex substrates (Syed,*et al*., 2010; Borrero, *et al*., 2011). This is proven in tomatoes. The Okungbowa and Edema (2006) and Okungbowa (2011) reports indicate the potential use of plant extracts for control of F. oxysporum.

1.2.2.3.2.Cultural control :

It can include tillage practices, flooding or heavy rain, polluted farm equipment and other cultural or environmental factors. Field-to-field propagation can occur when equipment and infected plants shift from one field to another. It is likely that macroconidia formed on the soil surface of the rotting vines could be blown short distances and help in spreading. Outside the total loss of crops, Other losses result from marketable yield losses, i.e. fruit that is formed but cannot be sold because it is too small, malformed, low in nutrients, or punctured or sunburned. There are also concealed losses arising from increased costs associated with shedding or replanting dead plants, fumigation or other soil treatments, and fuel and labor associated with the preparation of new land for cultivation once an existing field becomes unusable because it is infested with the fungus. Fusarium wilts are generally presumed to be monocyclic - that is, the disease does not exhibit plant-to-plant spread during the season (Egel and Martyn, 2007).

Cultivation of resistant varieties (the best solution) is commonly practiced and good hygiene (such as avoiding contamination, planting and seedling transplantation, irrigation and debris clearing from previous year's planting is maintained) (Jones and Woltz, 1981).

1.3 . Seeds dormancy:

1.3.1. Mechanism of Seeds dormancy

Seed dormancy in plants is a physiological process caused by external or internal causes and prevents germination of seeds even under ideal conditions. Dormancy of seed can be caused by conditions of rough seed coat, immature embryo, primitive embryo, and inhibitors. (Roholla,*at.al*.2011) , Factors affecting seed dormancy usually involve: seed coat (shell water and oxygen impermeability and mechanical resistance), embryos (depressed embryos, embryos being immature) and seed inhibitors. There are many ways to promote dormant seed germination (Latifi, 2001).

Dormancy in seeds throughout seed handling may be beneficial or problematic. The benefit is that it avoids the germination of seeds during storage and other handling processes, and the initiation of dormancy, for example by drying and dark storage, usually facilitates storage. However, seeds without dormancy such as recalcitrant seeds of rainforest species are very difficult to handle, for example. Since germination may start during transportation and temporary storage. On the other hand, where dormancy is complex and seeds need a very specific pre-treatment failure to resolve these problems, very poor germination can result. A low rate of seed germination that has proved to be sound and viable (Schmidt, 2000).

1.3.2. Classification system for seed dormancy:

(1) Physiological dormancy (PD) : The most abundant type of dormancy is present in gymnosperm seeds and all major angiosperm clades. It is the most common form of dormancy in temperate seed banks and the most abundant' in the field ' dormancy class. PD can be divided into three levels: deep, moderate, and no deep.). Seed dormancy is a

physiological phenomenon that many crops or wild plants are facing (Tajbakhsh & Ghiyasi, 2008).

(2) Morphological dormancy (MD): MD is evident in seeds with underdeveloped embryos (in terms of size), but differentiated (for example, in cotyledons and hypocotyl-radical seeds). These embryos are not dormant (physiologically), but they just need time to grow and germinate.

(3) Morphophysiological dormancy (MPD) : MPD is also evident in seeds with underdeveloped embryos, but their dormancy seems to have a physiological component. Such seeds also need a dormancy-breaking procedure, such as a given combination of warm and/or cold stratification, which can be replaced in some cases by GA treatment (Baskin & Baskin, 2004)..

(4) Physical dormancy (PY) : PY is caused by water-impermeable layers of seed or fruit coat palisade cells that control the movement of water. Mechanical or chemical scarifying can break the dormancy of PY. (Soltanpoor et al., 2009).

(5) Combinational dormancy (PY + PD) PY + PD is evident in seeds with water-impermeable coats (as in PY) combined with physiological embryo dormancy. (Tajbakhsh & Ghiyasi, 2008).

1.4. Hypothesis:

- There were no significant differences at the level of significance (P 0.05) between the different extract of *O. syriacum* and *S.fruticosa* leaves (chemical extract, essential oil and hydrosol) and the growth of two pathogenic fungi (*B. cinerea* and *F.oxysporum*).

1.5. Objectives :

- To evaluates antifungal activity against *B. cinerea* and *F. oxysporum* , of Sage (*S.fruticosa*) , Thyme (*O. syriacum*) from leave extract.
- In vitro test the essential oil from *S.fruticosa* , *O. syriacum* . antifungal activity against *B. cinerea* and *F. oxysporum*, and to evaluate its acute and sub chronic toxicological effects .
- To find alternatives of synthetic fungicides with natural non-pollutant ones, which are supposed to be safer , cheaper and friendly to environment and health.
- To examine seed germination for Sage (*Salvia fruticosa Mill*) and Thyme (*Origanum syriacum L.),* for achieving rapid, uniform and higher germination which will lead to higher plant stand establishment.

CHAPTER TWO : Literature Review

2.1.Bioactivity

Two words compose the term "bioactive": Bio-and -active. In etymology: bio-"bios"[bio-,-bio] from Greek, refers to: life. And –active from the Latin "activus," it means: fluid, full of fire, energy or action. This activity introduces all the phenomena from which a form of life, a working way or a method manifests itself ,The term "bioactive" is, in a strictly scientific sense, an alternative term for "biologically active"(Cammack. 2006)

A compound (or substance) that has biological activity if it affects a living organism directly. Depending on the substance, dose or bioavailability, those effects may be positive or negative, The term "bioactive compound" is not attributed to food-containing nutrients or, more broadly, nutrients that are essential to a living organism, such as primary metabolites. Controversies occur for food (or nutritional sources in general), except for the essential elements of the concept of bioactive compounds, with food constituents including water, carbohydrates, proteins, lipids, Such compounds have a wide range of effects, beginning with good health maintenance and even a therapeutic effect, or are harmful and even fatal. The dosage of bioactive compounds consumed is often important for whether the effect is positive or negative. (Aksel.2010). Bioactive compounds contain chemicals found in small amounts in plants [generally] and in certain foods (such as fruits, vegetables, nuts, oils and whole grains); they have actions in the body that can promote good health(Cammack. 2006)

For plants the word "food bioactive compound" usually does not include nutrients. Typical bioactive plant compounds are generated as secondary metabolites that are not necessary for the plant's daily operation (such as growth). It is then possible to define bioactive compounds in plants as secondary plant metabolites that cause pharmacological or toxicological effects in humans and animals.(Aksel.2010)

2.2 Natural products:

Plants produce a huge range of natural products with very complex structures. Unlike the "primary metabolites" which are important for plant growth and development, these products are commonly called "secondary metabolites." Formerly, secondary metabolites were considered "waste products" without any physiological role for the plant. Moreover, with the advent of the field of chemical ecology around 30 years ago, it became evident that these natural products perform important functions in the relationship of plants with their biotic and abiotic environment. For example, they may serve as compounds for the protection against herbivores and pathogens, as flower pigments which attract pollinators, or as hormones or signal molecules. Besides their physiological role in plants, natural products also have a strong effect on the human culture and have been used as condiments, pigments and pharmaceuticals throughout human history (Osbourn and Lanzotti,2009)

plants through scientific researchers have been found to contain valuable chemicals (Morrison and Boyd, 1987). These natural chemicals and their synthetic counterparts have continued to serve as feed stock in relevant industrial fields. While some are used in pharmaceutical, food, and chemical industry, others are applied as food flavors and fragrances, sweeteners, or even pesticides. Although western technologists have transformed many medicinal plants into more palatable forms like tablets, capsules, and syrups, many traditional healers still use plants in their crude form (herbal remedies). Extracts from some of the medicinal plants being used by traditional healers have been found to contain properties that inhibit the growth of bacteria, viruses, and other microbes (Ndubani & Hojer, 1999).

Aromatherapy has become more popular in recent decades which has sparked interest in essential oils. Lawless (1992) described" aromatherapy as a branch of alternative medicine

which claimed to have healing effects on the specific aromas carried by essential oils." A French chemist named Gattefosse first used the word aromatherapy in 1928. He worked perfume business for his families. (Lawless, 1992).

The family *Lamiaceae* has been extensively known to have immense medicinal, pharmacological and industrial properties. Many of these species within the *Lamiaceae* family has a potential of possessing essential oils which can be supplied to industry as raw material for different application in preparation of insecticides, antiseptics, per-fumes, spices and many other commodities (Buyisile and Magwa.2008).

2.3 Bio-pesticide:

Biopesticides are produced from natural living organisms such as animals, plants, and micro-organisms (e.g., bacteria, fungi, and viruses) that can control extreme plant-damaging insect pests through their non-toxic eco-friendly mode of actions, accordingly, it achieves importance throughout the world. Biopesticides and their by-products are used mainly for plant-injurious pest control (Mazid et al. 2011)

Biopesticides are categorized into three different categories: (1) defensive plant-incorporated, (2) microbial, and (3) biochemical. They have no residue issue, which is a matter of significant consumer concern, especially for edible fruits and vegetables. When used as a part of insect pest control, the effectiveness of biopesticides can be the same as that of conventional pesticides, particularly for crops such as fruits, vegetables, nuts and flowers. (Kumar 2012) Through combining the efficiency of synthetic pesticides with environmental safety, biopesticides work effectively with the tractability of minimal application limitations and with superior potential for resistance management .(Senthil-Nathan 2013).

Compared with chemical pesticides, biopesticides do not present the same regulatory problems seen with chemical pesticides. Biopesticides are often targeted specifically, are favorable to beneficial insects and do not cause environmental problems with air and water quality, and agricultural crops can also be reintroduced soon after treatment. Natural microorganisms can also be used in agricultural production , and human health risks are small. However, the use of biopesticides has several other advantages; for example, many target pests do not resist their effects.(Goettel *et al*. 2001 ; EPA 2006). Natural plant products are important source of new agrochemical for the control of plant diseases. So in recent years botanicals have become an alternative to pesticides and some of them already being used commercially in insect and pest management. Numerous plants have been found to possess phytochemical properties which are toxic to several plant pathogenic fungi. They not only provide effective control but also they are limited hazard to soil environment (Sungjemjungla,*et al*.2018).

Natural products derived from many plants have drawn scientific interest.(Cavaleiro& Pinto.2006) More recently, the antifungal properties of garlic-isolated allicin and ajoene (*Allium sativum*) have been shown.(Pyun & Shin.2006) Many essential oils have been believed to be effective against fungal pathogens in traditional medicine, although most of them are not clinical pathogens. Several authors have indicated that essential oils are among the most promising classes of natural compounds from which a new prototype of antifungal agents can be developed.(Kosalec & Pepeljnjak.2005) Work in this field may therefore lead to the development of effective drugs against pathogenic fungi.

2.4 Leaves extract of medicinal plant and their bioactivity :

In (Rechinger. 1982) study ,the genus Salvia, including some Iranian species, has been studied chemically and the presence has been reported of terpenoids, even the rare sesterterpenes, essential oils and flavonoids. Fifty-eight species of Salvia are found in Iran, 17 of which are endemic

In (Fatma *et.al*.2006) research, the aqueous extract of methanol from S aerial parts. Two new flavone glycosides, twelve known flavone glycosides, and chlorogenic acid were fractionated on a polyamide column. Two flavone aglycones and caffeic acid from the chloroform extract were also extracted and classified.

In (Honda & Koezuka.1988) analysis, α-pinene (7.6 percent to 4.3 percent), β-pinene (6.1 percent to 4.0 percent), and 1,8-cineole (39.2 percent to 20.3 percent) were found to contain the essential oils of S. aucheri subsp. aucheri from a separate locality in Turkey.

Za'atar has a very heavy yet pleasant aroma which its high content of essential oils gives to it. A recent study on the antifungal activity of essential oils of five tropical plant species, Apium leptophylum, Bidens formosa, Blumea decurrens, Conyza summasterensis and Tagetes minuta against the Aspergillus niger black mold stated The concentrations of any of these oils as low as 1 Ill / ml growth medium showed strong antifungal activity (Deans *et.al*. 1992)

A similar study was carried out on A. Parasiticus with essential oils from several plants; it has been shown that cumin and thyme oils developed a significant decrease in both mycelial growth and aflatoxin development when used at a concentration of 0.2 mg / ml and completely inhibited growth at higher levels. (Farag *et al*. 1989)

Another study performed on several Aspergillus species to determine the antifungal efficacy of mmjoram oil (from Origanum majorana L.) showed varying degrees of inhibition of the different molds, with A. Niger shows a greater degree of resistance to growth than A, flavus, A. Ochraceus and A. Parasitsisof resistance to growth than A, flavus, A. Ochraceus and A. Parasitsis (Deans & Svoboda. 1990)

The oil of marjoram was also studied along with the phenols thymol and carvacrol against Trichophyton concentricum, T. mentagraphytes and T. rub rum. In this investigation the agar overlay technique was used; the results obtained showed that all three organisms were strongly inhibited by the oil and by either one of the two phenolic compounds used (Sarer *et al*. 1985). Studies on Aspergillus parasiticus using thymol alone showed that a concentration of as low as 10 Ilg / ml in this mold induced a transitory growth inhibition (Buchanan & Shephard. 1981).

(Al-Masri, *et al*.2015) Investigated The effect of the native medicinal plant "Clammy Inula" (Inula viscosa L.) in combination with the low dose of the fungicide iprodione (Rovral ®) on the disease of gray moulds (*Botryis cinerea* Pers.) was tested in vitro and in live. The results revealed an antifungal effect of plant extract on mycelial growth rate and conidial germination of pathogen isolates.

The antifungal activity of the entire methanol extract and an ethanol fraction of three native Chilean plants (Ephe-dra breana, Fabiana imbricata, and Nolana sedifolia) were tested in vitro against Botrytis cinerea (the gray mold fungus) in (Sofia *et al*.2012). The results showed E . Breana and N. Sedifolia ethanolic fractions has fungal impact during 14 days , The complete methanolic fractions of the three studied plant species and the ethanolic fraction of F. imbricada did not show any fungicidal effect . Considering the antifungal

function of ethanolic extracts from E. Breana, N. sedifolia In vitro assay may be an interesting alternative for the control of *Botrytis cinerea* phytopathogen.

(Sharawi, et al. 2009) was investigated the impact of eighteen selected plant extracts native to Palestine :*Anthemis palestina, Artemisia herbaalba, Coridothymus capitatus, Inula viscosa, Majorana syriaca, Marticria chamomilla, Mintha piperita, Ocimmum basilicum, Origanum valgare, Paronychia argentea, Phagnalon rupestre, Rosemarinus officinalis, Salvia officinalis, Sinapis alba, Stachys distans, Teucrium polium, Thymus vulgaris, and Varthemia iphionoides*, against the pathogen and disease in vitro and in vivo. The results showed potent inhibitory effects on mycelial growth rate of *B. cinerea* isolates by all selected plant extracts tested. The strongest antifungal activity was observed with the extracts of I. *viscosa, M. syriaca, S. officinalis*, T. vulgaris and V. iphionoides, which inhibited the Mycelial growth rate of B. cinerea isolates by 76 - 100% at 4% concentration.

2.5 Essential oils:

2.5.1 EOs definition

Elsharkawy (2014) describes an "essential oil as a concentrated, hydrophobic liquid containing volatile compounds from plants". These oils are produced in the plant's green parts and are transported to other plant tissues for storage . It is then stored in small sacs in the roots, shoots, leaves, flowers ,hairs, trichomes and seeds of fragrant plants. (Galadima *et al*, 2012). Whitton (1995) explains that this oil gives the plant its distinctive smell which "help attract insects for pollination and allow the plant to protect itself from invading bacteria and fungi. We extend this protection to our benefit when we extract and use the oils because they can be antiseptic, bactericidal, viricidal and fungicidal as well as anti-inflammatory, anti-spasmodic, digestive or sedative depending on their individual plant

chemistry." Every oil extracted from various plants has a concentrated, distinctive combination of natural chemicals. (Whitton,1995).

2.5.2 Chemistry of EOs:

In general, Carbon, hydrogen and oxygen typically constitute the composition of essential oils that can be classified into two classes, oxygenated compounds and hydrocarbons. Examples of oxygenated compounds would include esters, alcohols, aldehydes, phenols, oxides, and ketones, while lactones, nitrogen, and sulfur compounds may sometimes also be present. Most of the hydrocarbons are terpenes (monoterpenes, diterpenes and sesquiterpenes). (Lawless, 1992)

Essintial oils constituents can be classified on to terpenoids and non-terpenoid hydrocarbons.

2.5.2.1 Non-terpenoid hydrocarbons :

Essential oils also contain non-terpenic compounds biogenerated by the phenylpropanoids pathway, such as eugenol, cinnamaldehyde, and safrole. phenylpropanoids originate through the shikimate pathway (Litchenthaler. 1999)

Although it forms a relatively small part of the essential oils, these aromatic compounds are an important group for the flavor and fragrance industry. The non-terpenoid group is composed of n-propyl benzene-derived constituents. The aromatic ring may hold dioxy groups of hydroxy, methoxy and methylene; the propyl side chain may contain hydroxyl or carboxy group (Shukla *et al*.2009)

2.5.2.2 Terpenes :

The main components of essential oil belong to the vast majority of the terpene family, several thousands of terpenic compounds have been identified in essential oils , such as functionalized alcohol derivatives (geraniol, α-bisabolol), ketones (menthone, p-vetivone) of aldehydes (citronellal, sinensal), esters (ÿ-tepinyl acetate, cedryl acetate), and phenols (thymol).(Modzelewska .2005).

Most of the oil constituents belong to the broad terpenic group. Terpenes usually refer to isoprene (2-methylbuya-1,3-diene) composed of hydrocarbon molecules. The unit of isoprene, which can build on it in different ways, is a molecule of five carbons. Two of the isoprene molecules contain monoterpenes, sisquiterpenes contain three isoprene molecules, four diterpenes, and five isoprene.(Swansom and Hohl 2006).

metabolic precursors and biosynthetic pathways involved in terpenoids are the mevalonate and mevalonate-independent (deoxyxylulose phosphate) pathways, (Figure 2.7).(Dewick, ,2002)

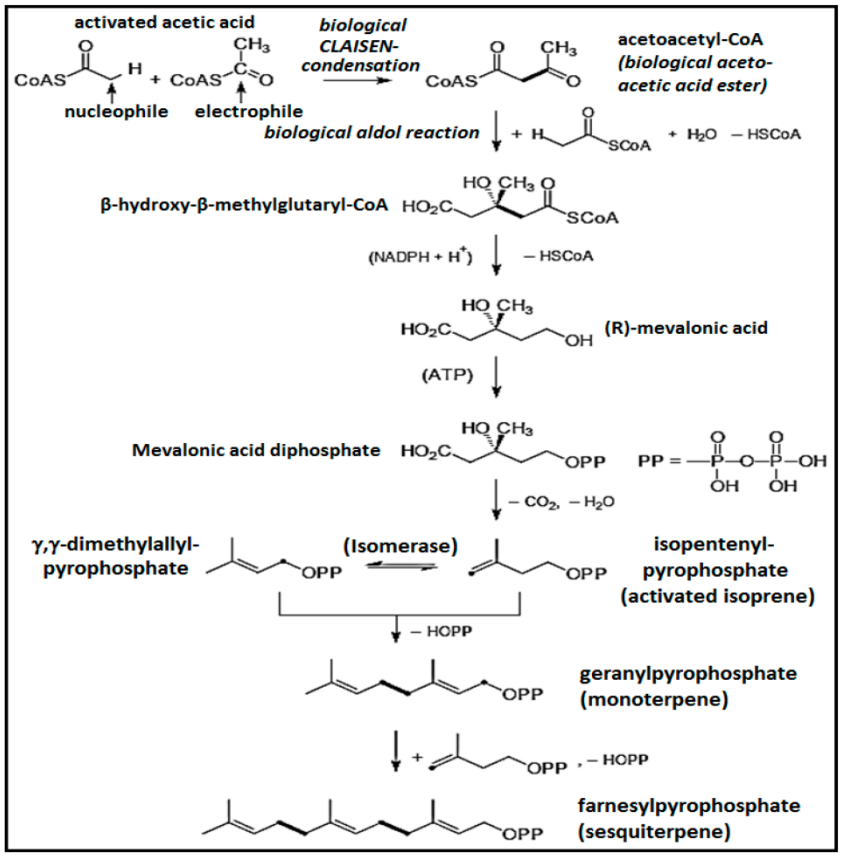


Figure 2.1: Biosynthesis pathways of monoterpenes and sesquiterpenes. (Dewick, ,2002)

2.5.3 Factors that affect the quality of essential oils

The composition of essential oils between various species of the same genus is clear, but the composition of oils from different organisms of the same species is influenced by many other factors. As well explained by Hussain (2009) ' ' There are numerous factors determining the composition and yield of the essential oil obtained. In some cases, these factors are difficult to separate, as many are interdependent and influence each other." One of the most important factors that can affect different chemo types of essential oils is the geo-climatic location. Some factors that may affect the plant's ratio of essential oil molecules include genetic variations, seasonal and maturity variations, the presence of fungal diseases and insects (Hussain, 2009). As the volatile content of the leaf increases with time and also with the size of the leaf, other considerations also include soil type,

plant life stage and even the time of day when harvesting is performed. (Bowles, 2003) Post-harvest drying and storage of the part of the plant used are also important factors which may influence the oil composition. (Hussain, 2009).

The methods of extraction used also influence the essential quality of the oil. For example, steam distillation can affect the chemical composition of essential oils, as heat and water vapor can cause molecular rearrangement, double bond hydrolysis, and generally produce substances that are not originally found in plants. (Bowles, 2003)

Another factor is the synthetic essential oils called "natural identical" oils vs. the natural oils. Currently, many oils and perfumes are produced almost entirely synthetically to be extracted from different plants. The perfumery and flavoring industries support these synthetic oils as they need stability in their products and any seasonal changes would lead to changes in the natural oils. Similar to the so-called" natural equivalent" products the naturally occurring essential oils have a completely different character . The synthetic oils are therefore much cheaper to produce than the genuine oils. Many aromatic oils contain a relatively low number of major components, several minor components, and a very huge number of trace elements. It would be virtually impossible to reconstruct such a complex mix of components including all trace elements . (Lawless, 1992)

2.5.4 Essential oil extraction methods

There are several methods to extract essential oils from plants. A compound's properties such as vapor pressure, solubility, polarity, and molecular size facilitate the isolation of essential oils. It is possible to isolate or extract essential oils using the following methods: effleurage, pneumatic process, maceration, voice, extraction of solvents, distillation procedures and liquid carbon dioxide method .(Trease and Evans, 1978).

Essential oils can be extracted by several ways such as distillation, solvent extraction, expression, maceration and enfleurage. (Whitton,1995)

2.5.4.1 Distillation

The simplest and most commonly used way of extracting the purest essential oils is distillation. The content of the plant is put in a flask and heated by water, steam or both. The heat causes the small oil bags to burst, releasing the volatile contents into the resulting vapor. Then the vapor is cooled by a condenser and once again becomes a liquid. The liquid is then split into the essential oil and fragrant water. (Whitton, 1995) This recondensed water is called hydrosol. The downside of steam distillation is that the extreme heat will quickly denature some delicate chemical components. (Whitton, 1995)

2.5.4.2 Solvent extraction

The plant material is put in a suitable solvent flask (e.g. hexane or supercritical carbon dioxide) and allowed to sit for a period of time (hours to days). (Bowles, 2003) .Whitton (1995) states the method further that " The resulting mixture is then filtered and becomes what is known as a ‘concrete’. The concrete is then mixed with alcohol, chilled, filtered and the alcohol evaporated off, leaving behind a highly perfumed oil which is called an ‘absolute’."

The disadvantage of this technique is that the extracted essential oil product which is ingested retains some of the solvent. This could be a problem since a reaction will occur if a consumer who is allergic to the solvent is applied to the skin. Solvent extraction is therefore typically used for oils that are used in perfumes and are not used on the skin. (Kububa, 2009).

2.5.4.3 Effleurage

The most important center for the extraction of flower oils is Grasse, in the South of France where the effleurage method is used and has its root. This method involves removing cold fat from the volatile oil. Glass plates are coated in the effleurage process with a thin layer of diluted fixed oil or fat on which the fresh flowers are spread. The essential oil passes gradually into the fat, removing the exhausted flowers and replacing them with a fresh supply until the fat is saturated with the volatile oil. The volatile oil is obtained in a fatty base during this process. Successive alcohol extractions are then performed. Alcoholic extracts can be put on the market as flower perfumes or as pure oil obtained by recovering the alcohol (Trease and Evans, 1978; EB, 1990).

2.5.4.4 Maceration

This involves immersing the flower in a melted fat at about 40 ° C to 80 ° C temperature. This method, similar to effleurage, takes between one and two hours for a shorter period. The volatile oil can be stored in fat or collected with alcohol in order to obtain the pure oil (EB, 1990).

2.5.4.5 Expression

Expression is used only on citrus oils. The peels are removed by hand or machine from the fruit and squeezed in order to release their oils.

2.5.5 Essential oils analysis:

The two key goals of the analysis of essential oils are (i) to recognize and measure as many components as possible. (ii) To assess the quality of the oils and to identify any possible adulteration that could affect their use . Gas chromatography GC (qualitative testing) and

gas chromatography-mass spectroscopy GC-MS (qualitative analysis) are typically used to analyze essential oils (Keravis ,1997). Testing of gas chromatography is a standard test of confirmation.

2.5.6 Uses of essential oils :

Essential oils are beneficial natural products used in many fields, such as perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition, insecticides. (Buchbauer 2000) . Essential oils in medicine are very important. Their medical practices are usually based not on scientific evidence but on historical purposes. Their uses range from skin treatments to cancer remedies (Elsharkawy, 2014) Inhaling essential oils or their internal volatile terpenes plays a major role in controlling the central nervous system. For example, aroma inhibiting essential oil from storax pills andpre-inhaling essential oils from Aconus gramineus rhizome are used in Chinese folk medicine for epilepsy treatment (Koo et al., 2003)

Clear in vitro evidence suggests that essential oil can function as an antibacterial agent against a broad range of pathogenic strains, including Listeria monocytogenes, Linnocua, Salmonella typhimurium, Shigella dysentria, Bacilluscerus, and Staphylococcus aureus. (Jirovetz et, 2005, Burt 2004, Dadalioglu and Evrendile 2004, Hulin et al., 1998)

It is stated that essential oils have insecticidal properties as an ovicidal, larvicidal, growth inhibitor (Isman et al., 1990). Also addressed was the effect of certain oils and their constituents on the reproduction of some species of insects and morphological changes in others (Smet et al., 1986). As a flavoring agent, essential oils are used. To improve their taste and aroma, flavors are added to the food. Vanilla flavoring is derived from vanilla beans and methyl salicylate, which has a distinctive taste and smell of minty. Natural

alternative to synthetic skin penetration enhancers can be recognized as essential oils and their terpene constituents. They are characterized by their relatively low price and promising activities that enhance penetration. The mechanism of skin penetration to improve terpene activity has been postulated (Barry 1991, Higaki et al., 2003)

2.6 Hydrosol

The essential oil vapors / constituents are kept in close contact with large volumes of steam / condensate water for prolonged time during distillation. Polar, oxygenated, odor-producing, hydrophilic, volatile oil components that can form hydrogen bonds with water are predominantly divided into the water process of distillation (Rajeswara *et al*. 2002). They are complex mixes containing large quantities of essential oil and other volatile, water-soluble secondary metabolites (Tannous et a1.,2004).Hydrosols with odor imparting, oxygenated, polar components of fragrant oils swept their way into global markets, especially in the west as inexpensive aromas, perfumery ingredients, aromatherapy products and caught the attention of global consumers aromatherapy practitioners, beauticians and scientists. Commercialization of hydrosols of different aromatic plant species. Adulterating costly hydrosols with water or alcohol isn't rare (Catty, 2001).

Floral and herbal waters are used in Europe, Asia and Africa for food flavoring and cooking. Thyme and oregano hydrosols are used as drinks and food ingredients, fresh and processed food products in India and other countries are flavored with rose water. Orange and rose flower hydrosols are used to taste household confectionary, Rose and orange floral hydrosols are also commonly used in cosmetics, soaps, toiletries and fragrances in Europe and Asia (Baser, 1992).
It’s also has an pesticidial effects ,as in indea some entrepreneurial farmers collect and

spray hydrosols on crops to repel insect pests and organism-causing diseases (Rajeswara Rao, 2012). Fungi toxic effects of many spice hydrosols against phytopathogenic fungi were reported (Boyraz *et al*. 2003). *Echinophora tenuifolio* and *Satureja hortensis* hydrosoLs (15%) effectively inhibited the growth (70-100%) of phytopathogenic fungi *Alterruaria citri, Botrytis cinerea, Fusarium oxysporum f. sp. tulipe* and *Rhizoctonia solani* for 7 days while the hydrosol (15%) of *Cumirlum cyminum* was partiatly effective (28%-80% inhibition in 7 days) against 3 fungi, ineffective against A. citri and, hydrosols of rosemary and basil were ineffectiv (Boyraz & Ozcan,2005)

Floral and herbal waters are used for medicinal purposes in Europe, Asia, and Africa. Rose and orange flower hydrosols are used as massage agents for skin care and their antiseptic properties(Catty,2001).

2.7 Chemical constituents of research's plants:

2.7.1 Sage , *S. fruticusa* :

Since ancient times, various species of the genus Salvia (Labiatae) have been used in folk medicine and undergo extensive pharmacognosic work aimed at identifying biologically active compounds (Pillips,1990). Species of Salvia contain different secondary metabolites such as sterols, sesquiterpenoids, sesterpenoids, diterpenoids, triterpenoids (Esquivel B, Sanchez.2000) essential oils, and flavonoids (Ulubelen & Topcu.1997). The aqueous methanol extract of the aerial parts of *S.fruticosa* Mill was fractionated on a polyamide column to afford two new flavone glycosides , twelve known flavone glycosides, and chlorogenic acid. Two flavone aglycones and caffeic acid . also were isolated and identified from the chloroform extract .(Mabry , Markham .1970). Chemical compositions of *S. fruticusa* oils were elucidated by GC/MS analysis (Table 2.5) .(Ashkun, *et al*.2010)

Table 2.1 The main compounds of essential oils of *S. fruticusa*.(Ashkun, *et al*.2010)

RRI	Compounds*	%
1032	**α-Pinene**	**5.8**
1076	Camphene	3.1
1118	β-Pinene	4.5
1174	Myrcene	3.8
1203	Limonene	2.1
1213	**1,8-cineole**	**52.8**
1280	*p*-Cymene	1.4
1437	α-Thujone	1.4
1451	β-Thujone	1.1
1532	**Camphor**	**5.8**
1612	β-Caryophyllene	2.1
1687	α-Humulene	2.6
1706	α-Terpineol	2.1
2008	Caryophyllene oxide	1.1
2104	Viridiflorol	1.1

* Only the percentages over 1% are indicated in this table.

2.7.2 Thyme , *O. syriacum*

The volatile oil of the Lebanese Za'atar (*Origanum syriacum* L.) was distinguished for its thymol and carvacrol; these two compounds constituted the major components of the oil and were present in the volatile oil extracted from the Origanum plant's leaves and shooting tips during the preflowering stage in the same proportion of 30 percent. After flowering and maturation, the amount of carvacrol in essential oil increased to 62%, while the concentration of thymol declined to 14%. For its antifungal activity against Aspergillus niger, Fusarium oxysporum, and Penicillium fungi, Origanum oil extracted from plants collected during the midseason was evaluated. The oil showed strong inhibitory action against the three fungi that had been tested. For the fungi tested, the oil's minimum inhibitory concentration (MIC) was found to be 0.1 µl / ml yeast extract sucrose broth. (rasha.1995) .GC and GC-MS studied the chemical composition of oil and found that it

contains 49.02% monoterpenes, 36.60% monoterpenes oxygenated and 12.59% sesquiterpenes. The main components are: ÿ-terpinene, carvacrol, p-cymene, and β-caryophyllene. The reduction in strength, antioxidant and 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity of essential oil .(Mehmet *et.al* .2003)

The chemical composition of Origanum syriacum L. grown in Turkey was 49.02% monoterpenes, 36.60% oxygenated monoterpenes and 12.59% sesquiterpenes.The major components were -terpinene, carvacrol, p-cymene and –caryophyllene (Alma et al. 2003). In the case of hydrodistilled essential oil from Origanum syriacum var. sinaicum cultivated in Egypt, thymol (24-29%), cis-sabinene hydrate (18-20%), - terpinene (13-15%), p-ymene (5-8%) and terpinen-4-ol (4-8%) were characterized as the main constituents (Baser et al. 2003) Carvacrol (78.4%) and thymol (17.9%) were the main abundant components of *O. syriacum* as the table 2.6 shows (Farhat, 2012)

Table 2.2 Chemical composition of essential oil of *O. syriacum*.(Farhat, 2012)

RI	Essential oil components	RT [min]	Content [%]
1190	Unknown	11.16	0.3
1249	Thymoquinone	12.44	2.5
1290	Thymol	13.18	17.9
1298	Carvacrol	13.39	78.4
1418	β-Caryophyllene	15.46	0.8

RI – Kovats index
RT – Retention time

2.8 Mechanisms of seed dormancy :

Having one type of dormancy, the seeds of many medicinal and grassland plants in natural habitats ensure their survival for many years, but for propagation and cultivation of these plants it is essential to release the seed for germination (Tajbakhsh, 1996; Javanmiri Pour

et. al., 2013). Considering the importance of medicinal plants for treatment of different disease, and also limited natural habitats and slightly breeding, planning of their cultivation and domestication is necessary (Soltanpoor et al., 2009).

Hejabi & Soltanipoor (2006) have shown that pre-eruptive mechanical scarification can influence Salvia mirzayanii's germination characteristics to increase the germination of this medicinal plant.

Almoshiri *et.al*. (2009) also concluded that Teucrium polium seed germination is substantially increased after sandpaper treatment compared with untreated seed.To promote sprouting of T. Polium, Kochacki & Azizi (2005) found that 500 ppm gibberellic acid treatment had the most positive effect on germination of seeds.

Further experiments of hot water treatment have been done showing that this type of treatment has had an effect on seed germination of many medicinal plants and rangelands. It has been shown the treatment of warm water with S. Seed germination was significantly influenced by the mirzayanii (Hejabi & Soltanipoor, 2006). Cooling treatment at 5 ° C improved germination of the Ferula gomussa for 2 weeks (Macchai *et.al*., 2001).

Chapter three: Methodology

The research was conducted in three parts:

First Experiment : Testing the bioactivity of Sage , Thyme leaves extract against *B. cinerea and F.oxysporum*

Second experiment: Essential oil isolation and testing their bioactivity on *B. cinerea and F.oxysporum*

Third experiment : Germination of Sage, Thyme seeds by different treatments.

3.1. First Experiment : Testing the bioactivity of Sage , Thyme leaves extract against *B. cinerea* and *F.oxysporum*

3.1.1 Sample Collection:

Mature Sage and Thyme leaves were collected in 2019 from different location in Bani Na'im which located 8 kilometers east of Hebron in the Hebron Governorate

3.1.2 Sample preparation

sage and thyme leaves shaded, leaves samples were washed with water to eliminate any traces of dust. In order to stabilize the byproduct, the leaves were shade air-dried at ambient temperature to reduce their moisture content to prevent microbial fermentation and subsequent degradation, the dried leaves were a grind to get fine powdered material. The powder was stored in plastic containers at room temperature away from the sun for use

3.1.3 Simple green extraction method (Extraction of leaves) :

50 grams of two plants leave powder were macerated in 500 ml ethanol(99%) at 40°C and soeked for 24 hours. The extracts then filtered through a Whatman filter paper. The supernatants were combined and concentrated on a rotary evaporator at 40°C under vacuum to get a paste of *S.fruticusa and O. syriacum* leaves extract. (at AlQuds University

laboratory as shown in figure 3.1).The resulting crude plants leaves extract was stored at -4 °C in dark bottles.These procedure applied to all samples.

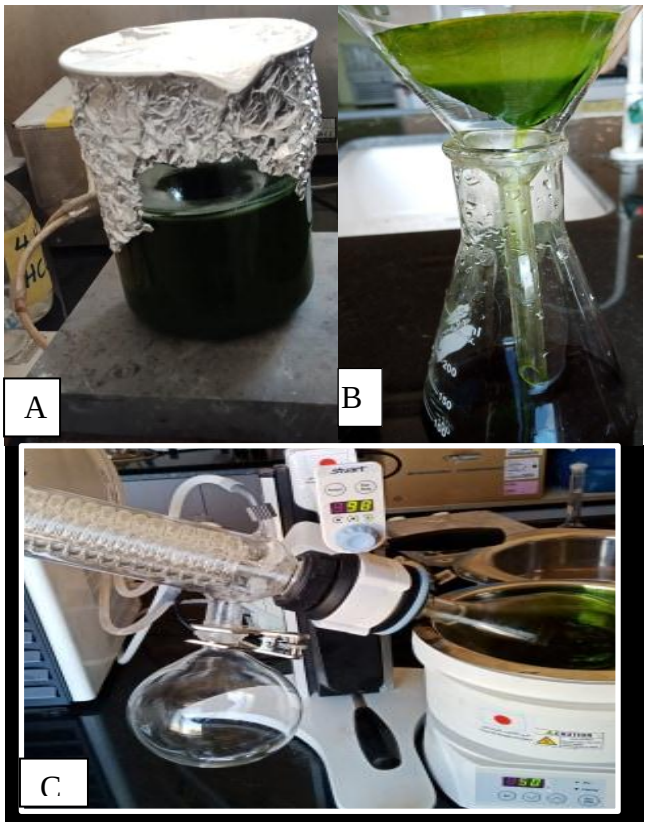


Figure 3.1:(A) leaves extract on magnetic stirrer.(B) extract filtration by Whatman filter paper .(C) Evaporation of leaves extract using a rotary evaporator.

3.1.4 In Vitro Assays (Mycelial Growth Rate)

Mycelia Growth Rate used to evaluate the antifungal activity of both plants leaves extract, and it used to study its effect on different concentration .The effect of Sage (S.fruticosa) , Thyme (O. syriacum) leaves extract on mycelial growth rate of the both fungi were evaluated in vitro using 4g potatoes dextrose agar (PDA) medium amended with 0.1 g chloramphenicol. Each Flasks containing 100 ml of distilled water and 4g of PDA medium

are placed on hot plate with magnetic stirrer to dissolve and homogenize the components; flasks are then autoclaved and allowed to cool down to 55˚C - 60˚C.

The extract prepared and added to the growth media to give final concentrations of plant extract 0%, 1%, 2%, 3% .Growth media (14 ml) are dispensed into each Petri plate (90 mm diameter). The experimental design was completely randomized (CRD) with five Petri plates (replicates) for each plant extract concentration for each *B. cinerea* isolate and *F.oxysporum* .Amended Petri plates were then inoculated with 5 mm mycelium disks from 5-day-old cultures of the B. cinerea and *F.oxysporum* isolates (figure 3.2). Plates were then incubated in growth chamber at 25˚C. Colony diameters were measured after 3 days and 6 days and the mycelium growth rate (MGR, cm2 ·day−1) was calculated using the following equation:

R = {(D/2) 2*л - (d/2) 2 *л }/T ……..(1) (Barakat and Al-Masri, 2005).

Where،

R: mycelium growth rate.

D: average diameter of colony (cm) after 6 days

d: average diameter of colony (cm) after 3 days ،

л :3.14

T: time of incubation (day)

Figure 3.2: Preparing of PDA media and cultivation of both pathogens.

3.2. Second experiment: Essential oil isolation and testing their bioactivity on *B. cinerea and F.oxysporum*

3.2.1. Plant material .

For the extraction of essential oils of *S.fruticosa,*and *O. syriacum* were collected locally from different origin in Hebron then has been deposited in the herbarium of science department of al-Quds University .

3.2.2. Isolation of essential oil:

A distillation by Clevenger apparatus:

For the steam distillation two different setups were used. The first setup used for steam distillation is a Clevenger apparatus to which a false bottom extraction container is connected at the bottom of the apparatus and filled with leaves powder. The steam formed by heating the distilled water in the round bottom flask has to move through the extraction container where it causes the volatile components from the leaves to evaporate and travel

with the steam through the connecting tube on the Clevenger apparatus to the condenser then to the receiver tube. The function of the rubber tube is to recycle the water back into the round bottomflask when the receiver tube is too full.

Air-dried plant materials (100 g) were placed in a 5 l round-bottom distillation flask and 3 l double distilled water was added. The essential oils were obtained by steam distillation for 3 h using Clevenger-type apparatus. The isolated fractions of plant parts exhibited two distinct layers—an upper oily layer and the lower aqueous layer(as figure 3.3 shows). Both the layers were separated and, after removing water traces with the help of capillary tubes and anhydrous sodium sulphate, the essential oils were stored at 4 °C in a clean amber glass bottle until used .

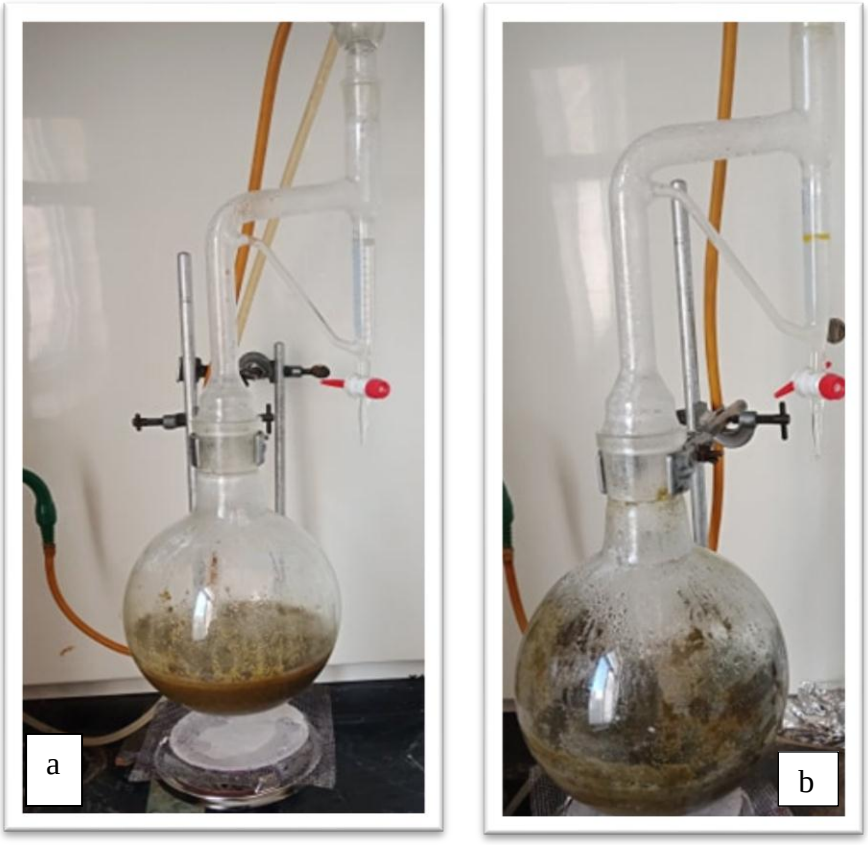


Figure 3.3: The isolated fractions of plant parts exhibited two distinct layers;EOs & hydrosol(a) *S.fruticosa*, (b) *O. syriacum* .

The yields of essential oil of sage and thyme were expressed in g relative to 100 g of dry vegetable matter; it was calculated according to Equation (2):

Yield (%) = amount of extracted oil (g)/ amount of dry vegetal matter mass (g)……...(2)

Yield for Thyme (%) = 1 g thyme extracted oil/ 100 g of dry vegetal matter mass = 1%

Yield for Sage (%) = 1 g sage extracted oil/ 100 g of dry vegetal matter mass = 1%

The yields of hydrosol of sage and thyme were expressed in g relative to 100 g of dry vegetable matter , both sage and thyme gave from 100 g of dry vegetal matter mass 30g of hydrosol

3.2.3 In Vitro Assays (Mycelial Growth Rate) :

The concentrations of the essential oils were prepared by dissolving ,5 ml of each one of the essential oils in 10 ml of 70% ethanol to obtain .5 % ,1%, 2% and 4% concentrations for sage essential oils and .5 % ,1%and 2% for thyme essential oils and 0% which is the control for both plants ,and added to the growth media .

The hydrosol concentration were also prepared and added to the growth media to give final concentrations of 0%, 1%, 2%, 3% .

Growth media (20ml) are dispensed into each Petri plate (90 mm diameter). The experimental design was completely randomized (CRD) with five Petri plates (replicates) for each essential oils and hydrosol concentration for each fungi . Amended Petri plates were then inoculated with 5 mm mycelium disks from 5-day-old cultures of both isolates. Plates were then incubated in incubator at 25˚C. Colony diameters were measured after 3 days and 6 days and the mycelium growth rate (MGR, $cm^2 \cdot day{-}1$).

3.3 Minimum inhibition concentration (MIC) :

After the result of thyme experiment we decided to repeat the experiment to find the minimum inhibition concentration that the fungi can grow on the PDA amended with chemical extract and essential oil of thyme , by doing the same procedure except the concentrations which changed to lowest concentration as possible as we can (.001%, .01% ,and .1%)

3.4 Statistical analysis :

The readings were taken from the experiments and analyzed by sigma stat program by one way Analysis of Variance (ANOVA) to find the mean desperation and significant difference , using fisher LSD test (least significant different test) .

3.5 Third experiment : Germination of Sage, Thyme seeds by different treatments.

3.5.1.seeds collection

Collect the seeds of the selected plants for research from several sites and cassations in the West Bank in Palestine between October and November 2018 , so they are collected from healthy mother plant.

The seeds are purified in laboratory by seed blower machine which is a batch system very suitable for cleaning very small seed lots. Then store them at room temperature until processed by treatments before planting.

3.5.2 Methods for breaking dormancy:

3.5.2.1 Seed treatments:

Based on the previous studies and their results, the best treatments suitable for medicinal plants which gives higher germination rate were selected to be performed in this research :

a.Scrape with sandpaper

In order to perform scrape treatment, the seed hulls by sandpaper (between two layers of sandpaper) and pulverized until thinning of their shells. After performing the scrape treatment, the seeds were placed in water for 24 hours. After this period of time, treated seeds were transferred in a culture container containing wet filter paper and were kept at temperature of 25 ± 3 ° C .

b.Growth regulator treatment (gibberellin, kinetin)

the seeds were soaked in water for 48 hours. Then the seeds were put in a container, and 500 ppm gibberellic acid or kinetin solution was added. In these conditions they were kept for 24 hours and 48 hours . After this treatment, the seeds were washed with water and then transferred to a container culture.

c. Chilling

In the chilling treatment, the seeds were soaked in water for 48 hours and then were maintained for 72 h in moist sterile plastic bags at 4°C. The seeds were kept steadily moist in cold. After the specified time, the seeds were placed to germinate at room temperature.

d. Control treatment

The seeds were placed in a container culture and irrigating daily with distilled water to maintain the moisture.

3.5.3 Seed germination

After treatment, the seeds were cultivated within the planting bed and placed on filter paper. Germination counting was started from the third day of planting and was performed daily for 4weeks. While supplying adequate moisture ,germination changes were recorded

according to the special forms of viability. Results were observed daily and the number of seeds in each treatment was recorded. All germinated seeds were counted and removed from the container. After performing the all steps within the prescribed time, germinated seeds and germination percentage were calculated by using the following formula according to the method Panwar (Panwar & Hardwaj, 2005):

Germination rate = n/N*100 (%)……..(3) (Panwar & Hardwaj, 2005)

where n= total number of germinated seeds during the period,

N= total number of planted seeds.

3.5.4 Summary of study design and data analysis :

To evaluate the effect of different treatments on seed germination of *S.fruticosa & O. syriacum* collected between October and November in 2018 , we have performed completely randomized experimental tests with 3 replications **.** Data analysis was performed using sigma stat software through analysis of variance (one way ANOVA) and Duncan test to compare the means.

Chapter four : Result and discussion

4.1 In Vitro Assays :

This section shows the results of the effect examination of *S.fruticosa & O. syriacum* leaves extract , EOs and hydrosol against *B. cinerea* and *F. oxysporum* in vivo which includes the mycelia growth rate at different concentration of them .

4.1.1 Result of Sage *S. fruticusa:*

The effect of *S.fruticosa* leaves extract , EOs and hydrosol on mycelia growth rate of *B. cinerea* and *F. oxysporum* were evaluated in vitro by using potatoes dextrose agar (PDA)medium. *S.fruticosa* leaves extract , EOs were examined, for each one of them five media samples were prepared with different concentrations, the volume of each sample was 100 ml .

The results showed that *S.fruticosa* leaves extract , EOs and hydrosol had an antifungal effect on mycelia growth of *B. cinerea* and *F. oxysporum* pathogens. The leaves extract at the concentration of 2% -3 % reduced the mycelium growth of *F. oxysporum* . and reduce the mycelium growth of *B. cinerea* at the concentration 1%-2% and totally inhibited(100%) at 3% concentration. But *F. oxysporum* growth decline gradually when adding salvia leave extract at 1, 2 and 3% .The reduction of mycelium growth was positively correlated with increasing extract concentrations. The percentage of reduction of mycelium growth ranged between for *B. cinerea* 42% - 100% ,and 12%-38% for *F. oxysporum* depending on concentrations. The highest area of both fungi growth at 0.0% (without adding any concentration of extract), then begin to decline gradually when adding salvia leave extract at 1% and 2% until the growth of *B. cinerea* finally stopped at concentration 3% .but *F. oxysporum* growth decline gradually when adding salvia leave extract at 1, 2 and 3% .

S.fruticosa EOs at the concentration of 1% -2 % reduced the mycelium growth of *F. oxysporum*, and at 4% its totally inhibited(100%) its growth . and reduce the mycelium growth of *B. cinerea* at the concentration 1%-2% and totally inhibited(100%) at 4% concentration The reduction of mycelium growth was positively correlated with increasing EOs concentrations. The percentage of reduction of mycelium growth ranged between for *B. cinerea* 22% - 100% ,and 24%-100% for *F. oxysporum* depending on concentrations. The highest area of both fungi growth at 0.0% (without adding any concentration of EOs), then begin to decline gradually when adding salvia EOs at 0.5% - 2% until the growth of *B. cinerea* finally stopped at concentration 4% .but *F. oxysporum* growth decline gradually when adding salvia EOs at 0.5- 2% and stop at 4% as showing in figure 4.1.The results correspond with Topcu, *et al*.2013 study which showed that *S.fruticosa* EOs have antifungal action .Also, results compatible with Yilar, *et al*.2018 study which showed that the antifungal activity of salvia oils on different plant pathogens.

S.fruticosa hydrosol concentrations 1%,2%and 3% reduced the mycelium growth rate (MGR) of both pathogens isolates grown on PDA amended medium compared to the control. As the concentrations increased the total inhibition on MGR were increased. MGR reduction was positively correlated with increasing *S.fruticosa* hydrosol concentrations. The percentage of reduction of mycelium growth ranged between for *B. cinerea* 39% - 51% ,and 17%- 44% for *F. oxysporum* depending on concentrations. .The highest area of both fungi growth at 0.0% (without adding any concentration of hydrosol), then begin to decline gradually when adding salvia hydrosol at 1% - 3% as showing in figure 4.1 . *F. oxysporum* growth decline gradually when adding hydrosol at 1%-3% as showing in figure 4.2.The results correspond with Boyraz et al. (2003) that Fungi toxic effects of many spice hydrosols against phytopathogenic fungi.

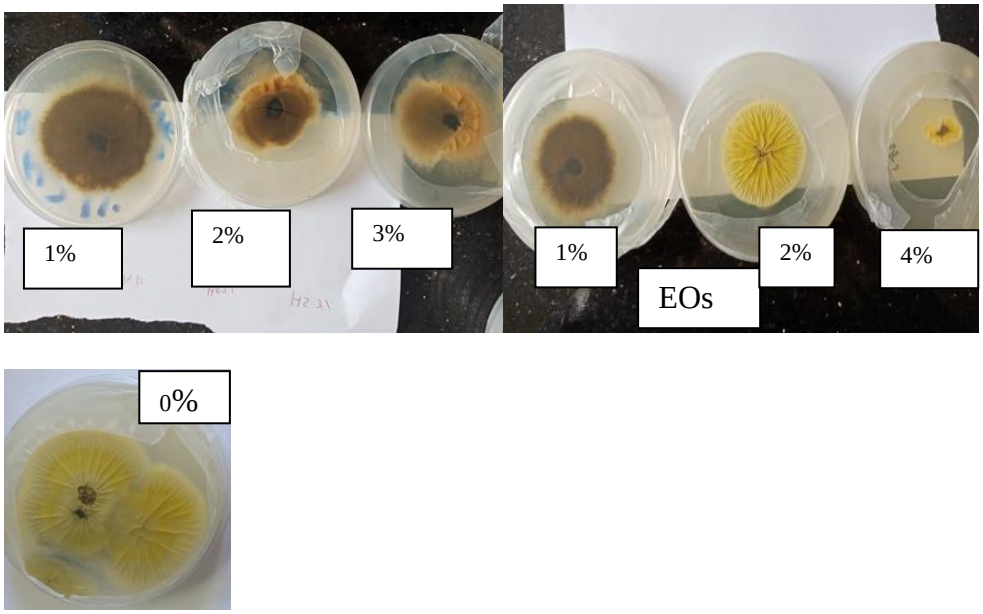


Figure 4.1: Effect of *S.fruticosa* EOs & hydrosol on Mycelium Growth of *B. cinerea* at different concentrations.

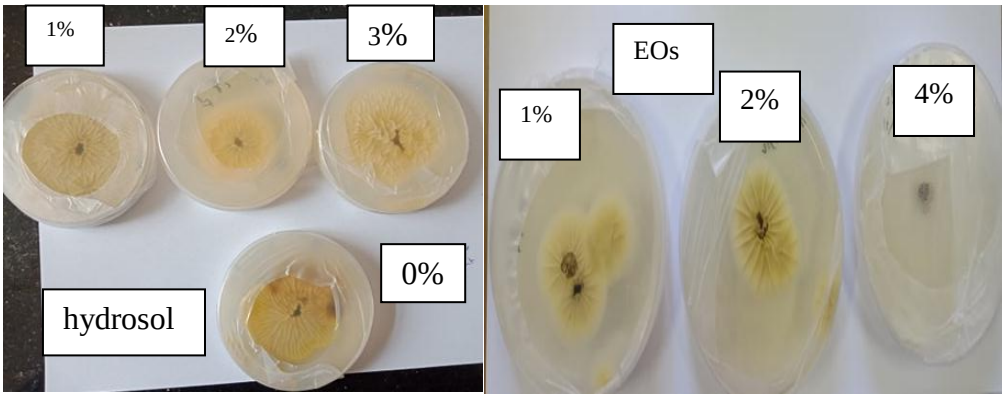


Figure 4.2: Effect of *S.fruticosa* EOs & hydrosol on Mycelium Growth of *F. oxysporum* at different concentrations.

Table 4.1: Effect of *S.fruticosa* on the Mycelium Growth Rate (R) against *B. cinerea* and *F. oxysporum* .

Fungi R**	**Sage,** *S.fruticosa*						
Chemical extraction							
concentrations	0%*	1%	2%	3%		F	Fisher test(LSD)
F. oxysporum	137.6a	120.8a	76.1a	85a		1.02	-----
B. cinerea	97.4 a**	56.5 b	28.5 c	0 cd		16.8	30.7
Essential oil							
concentrations	0%	0.50%	1%	2.00%	4%		
F . oxysporum	137.6 a	104.7 a	63 b	33.5 b	0 b c	5.4	71.2
B. cinerea	97.4 a	75.9 a	44.5 b	36.1 b	0 b c	6.03	45.8
hydrosol							
concentrations	0%	1%	2%	3%			
F. oxysporum	173.1a	143.6a	135.2a	95.7a		.676	-----
B. cinerea	170.1a	102.9a	84.8a	82.1a		.967	-----

*Results are expressed as average of six values.
**Different small letters within column indicate significant difference ($p < 0.05$, $n = 5$)
**R: mycelium growth rate (R = {(D/2) 2*л - (d/2) 2*л }/T).
D: average diameter of colony (mm) after 3days.
d: average diameter of colony (mm) after 6days.
Fisher's Least Significant Difference (LSD) test.: after one-way ANOVA, to compare the mean of one group with the mean of another .

As shown in table 4.1 and figure 4.3, there are small significant differences in the rate of reduction from *S.fruticosa* leaves extract against F. *oxysporum* due to the fact of an increase in the effect of blocking effect of plant extracts on *F. oxysporum* was observed depending on the dosage increase and extract used(Yılar,2016), while there are a significant differences from *S.fruticosa* leaves extract against *B. cinerea.*

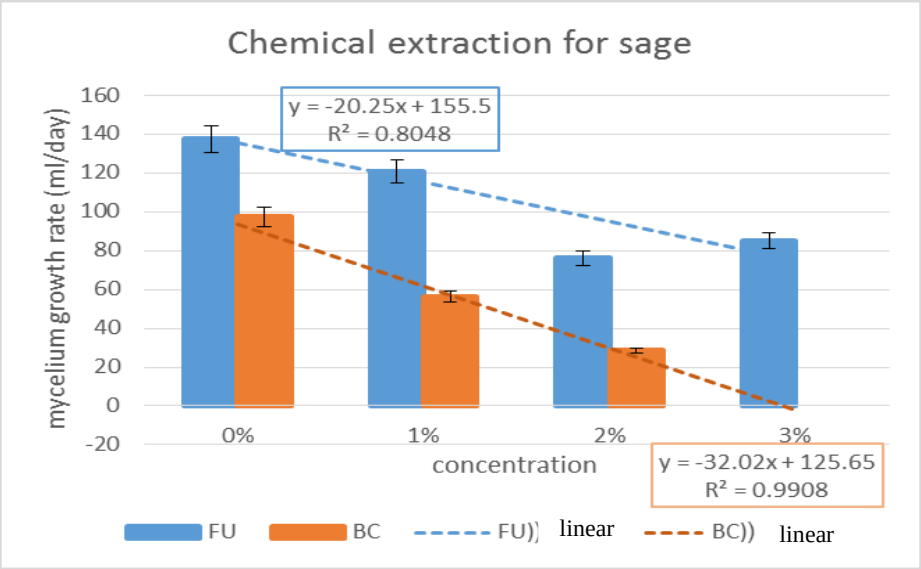


Figure 4.3 : Effect of *S.fruticosa* leaves extract in different concentrations on the Mycelium Growth Rate (R) against *B. cinerea* and *F. oxysporum* .

Statistical analyses showed that there is significant differences in the rate of reduction (R) as the concentration of leaves extract increases from 1 to 2, and 3% indicated by small letters (a, b, c,and d). except at 3% of *S.fruticosa* leaves extract aginst *B. cinerea* were no significant differences in the rate of reduction observed as there is complete reduction (R is zero at 3%). On the other hand, statistical analyses showed that there is significant differences in the rate of reduction (R) as the concentration of *S.fruticosa* increases from 0.5to1, 2, and 4% indicated by small letters (a, b, c,and d). except at 4% of *S.fruticosa* essential oils aginst *B. cinerea* were no significant differences in the rate of reduction

observed as there is complete reduction (R is zero at 4%) against both fungi as the Figure 4.4 shows .

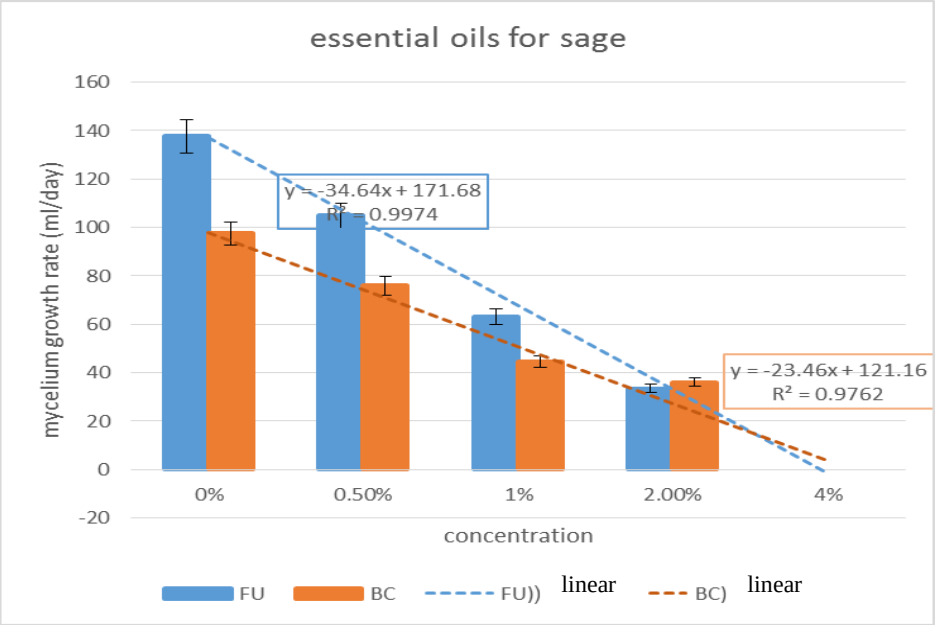


Figure 4.4 : Effect of *S.fruticosa* EOs in different concentrations on the Mycelium Growth Rate (R) against *B. cinerea* and *F. oxysporum* .

However, *S.fruticosa* hydrosol wasn't significant in the the rate of reduction (R) of *F. oxysporum*, The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference , the highest concentration used was 3% reduce MGR of *F. oxysporum* by 44% as figure 4.5 shows. Several studies have proven the efficacy of hydrosol at high concentrations, hydrosol (15%) of Turkish *Satureja hortensis* was fungicidal to the mycelial growth of Botrytis cinerea. The essential oil was less effective (Boyraz & Ozcan,2005).

R^2 equation in the figures below represent the regression coefficients , as r^2 was more than 0.6 *S.fruticosa* extract ,EOs and hydrosol were active on both pathogenic fungi.

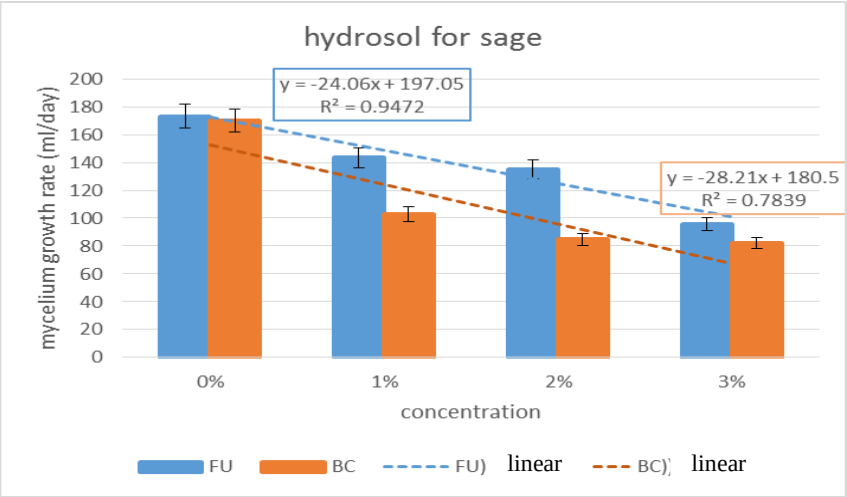


Figure 4.5 : Effect of *S.fruticosa* hydrosol in different concentrations on the Mycelium Growth Rate (R) against *B. cinerea* and *F. oxysporum*

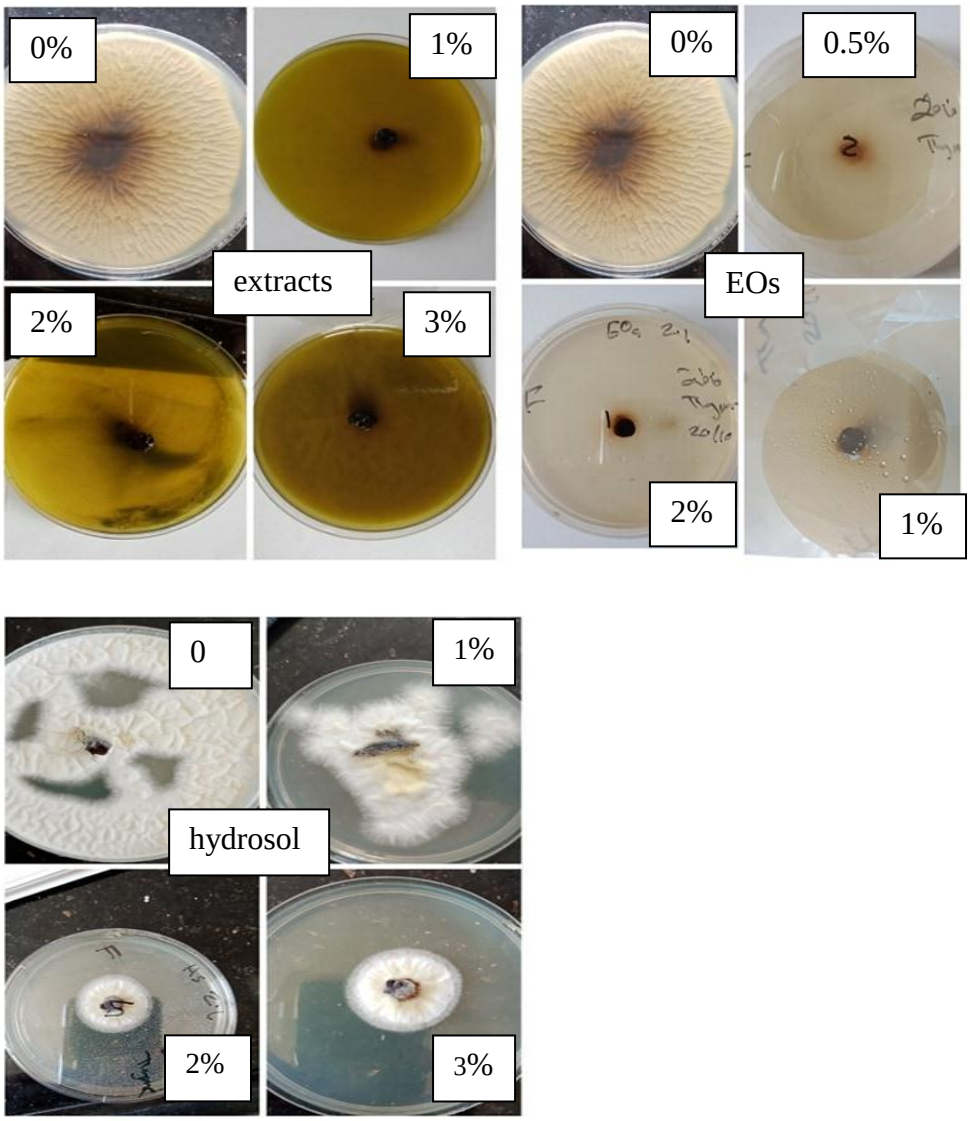
4.1.2.Result of Thyme ,*O. syriacum* :

The effect of *O. syriacum* leaves extract , EOs and hydrosol on mycelia growth rate of B. cinerea and *F. oxysporum* were evaluated in vitro by using potatoes dextrose agar (PDA)medium. *O. syriacum* leaves extract , EOs were examined, for each one of them five media samples were prepared with different concentrations, the volume of each sample was 100 ml .

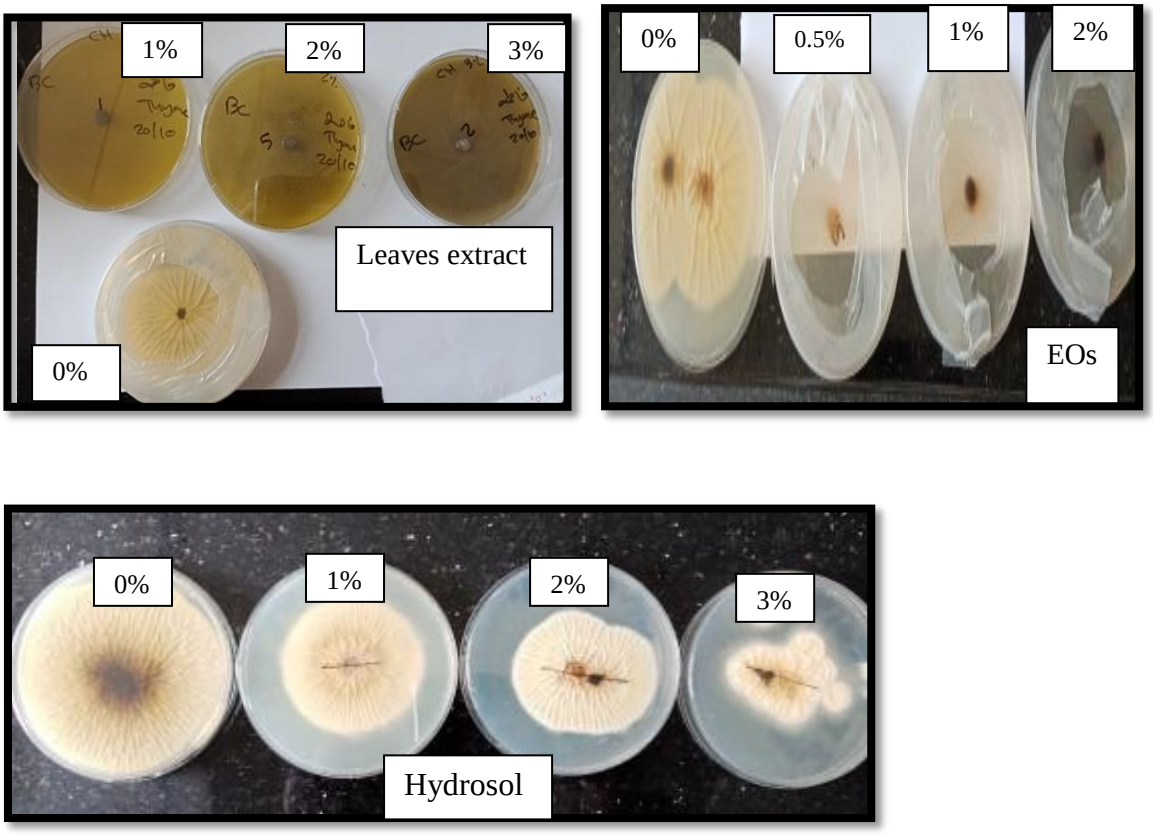
The results showed that *O. syriacum* leaves extract , EOs and hydrosol had an antifungal effect on mycelia growth of *B. cinerea* and *F. oxysporum* pathogens. The leaves extract at the concentration of 1% -3 % inhibited the mycelium growth of *F. oxysporum(*as showing in figure 4.6.) and *B. cinerea (*as showing in figure 4.7.) .The reduction of mycelium growth was positively correlated with increasing extract concentrations . *O. syriacum* EOs was strongly effective on B. cinerea and *F. oxysporum* because its shows 100% inhibition on the mycelium growth rate (R) .and the reduction of mycelium

growth was positively correlated with increasing EOs concentrations on F. oxysporum(as showing in figure 4.6.) and B. cinerea (as showing in figure 4.7.).

O. syriacum hydrosol concentrations 1%,2%and 3% reduced the mycelium growth rate (MGR) of both pathogens isolates grown on PDA amended medium compared to the control. As the concentrations increased the total inhibition on MGR were increased. MGR reduction was positively correlated with increasing *O. syriacum* hydrosol concentrations on *F. oxysporum* .The percentage of reduction of mycelium growth ranged between for B. cinerea 38% ,and 40%- 88% for F. oxysporum depending on concentrations. .The highest area of both fungi growth at 0.0% (without adding any concentration of hydrosol), then begin to decline gradually when adding *O. syriacum* hydrosol at 1% - 3% of *F. oxysporum (*as showing in figure 4.6.) and *B. cinerea (*as showing in figure 4.7.).The results correspond with Boyraz *et al.* (2003) that Fungitoxic effects of many spice hydrosols against phytopathogenic fungi. Also , hydrosols of lavender(*Lauandula stoechas*), thyme (*Thymus vulgaris*) showed strong activity against wood decay brown rot (*Coniophora puteana, Gloeophyllum trabeum, Oligoporou. s placenta*) and white rot (*Ceriporiopsis subuermisphora, Phanerochaete chrysosporuim, Pleurotus ostreatus, Trametes uersicolor*) fungi according to Sen & Yalgin(2010) study.



Figuer 4.6: Effect of *O. syriacum* leaves extract ,EOs & hydrosol on Mycelium Growth of *F. oxysporum* at different concentrations.



Figuer 4.7: Effect of *O. syriacum* leaves extract, EOs & hydrosol on Mycelium Growth of *B. cinerea* at different concentrations.

In primarily experiment, the result revealed that *O. syriacum* extract was completely inhibited the mycelium growth rate of both pathogens grown on potato dexatros agar amended with chemical extract at concentration(1%,2% and 3%) . furthermore, *O. syriacum* essential oils completely inhibited the mycelium growth rate of both pathogens grown on PDA amended with *O. syriacum* essential oils at concentration(0.5% , 1% and 2%) .So in repeated experiment concentrations that used were the least possible (.001% , .01% and .1%) of *O. syriacum* extract and essential oils that . which gave less effect of them on both pathogen *F.oxysporum and B.cinerea*.

O. syriacum chemical extract was significant at concentration > .001% with significant difference between concentration .01% and .1% . at the concentration 0.1% the reduction

of MGR on grown *F. oxysporum* on PDA amended with concentration was 52% . Furthermore *O. syriacum* chemical extract wasn't significant at concentration > .001% . at the concentration 0.1% the reduction of MGR on grown *B. cinerea* on PDA amended concentration was 27% which mean it didn't highly effect on *B. cinerea*.

While *O. syriacum* essential oil was significant at concentration > .001% with significant difference between concentration .01% and .1% . at the concentration 0.1% the reduction of MGR of *F.oxysporum* were 48%. however *O. syriacum* essential oil was significant at concentration > .001% with significant difference between concentration .01% and .1% . at the concentration 0.1% the reduction of MGR of *B. cinerea* were 3%.

Table 4.2: Effect of *O. syriacum* on the Mycelium Growth Rate (R) against *B. cinerea* and *F. oxysporum* .

Fungi R**	**Thyme,** O. syriacum					
	Chemical extraction					
Concentrations	0%*	1%	2%	3%	F	Fisher test(LSD)
F. oxysporum	171.6 a**	0 b	0 b	0 b	48.7	39.7
B. cinerea	177 a	0 b	0 b	0 b	42.2	44.1
	Essential oil					
Concentrations	0%*	0.50%	1%	2.00%		
F. oxysporum	171.6 a	0 b	0 b	0 b	48.7	39.7
B. cinerea	177 a	0 b	0 b	0 b	42.2	44.1

	hydrosol					
Concentrations	0%	1%	2%	3%		
F. oxysporum	171.6a	234.2a	149a	77.1a	1.4	-----
B. cinerea	252.4a	366.7a	213.9a	158.5a	1.65	-----
	Essential oil (MIC)					
Concentrations	0%	0.001%	0.010%	0.100%		
F. oxysporum	171.6 a	107.2 b	81.5 b	88.9 b	5.5	55.3
B. cinerea	177.5 a	110.6 b	98.4 b	78.7 b	3.9	68.2
	CHEMICLA EXTRACTION (MIC)					
Concentrations	0%	0.001%	0.010%	0.100%		
F. oxysporum	171.6 a	94.1 b	92.9 b	82.6 b	3.5	70.3
B. cinerea	177.5a	183.2a	109.9a	85.7a	2.3	-------

*Results are expressed as average of six values.

**Different small letters within column indicate significant difference ($p < 0.05$, $n = 5$)

**R: mycelium growth rate (R = {(D/2) 2*л - (d/2) 2*л }/T).

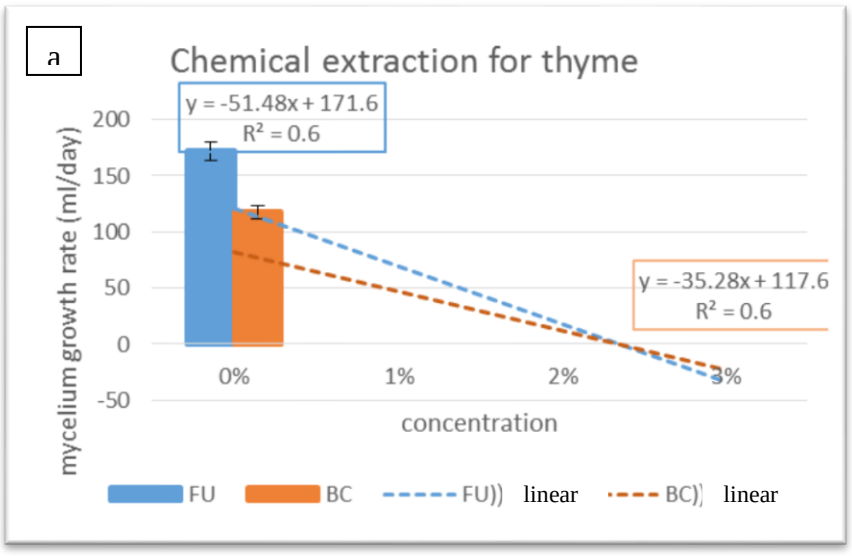
D: average diameter of colony (mm) after 3days.

d: average diameter of colony (mm) after 6days.

Fisher's Least Significant Difference (LSD) test.: after one-way ANOVA, to compare the mean of one group with the mean of another

As shown in table 4.2 and figure 4.8, there are significant differences in the rate of reduction from *O. syriacum* leaves extract against F. *oxysporum* and *B. cinerea.*

R^2 equation in the figures below represent the regression coefficients , as r^2 was more than 0.6 S.fruticosa extract ,EOs and hydrosol were active on both pathogenic fungi.



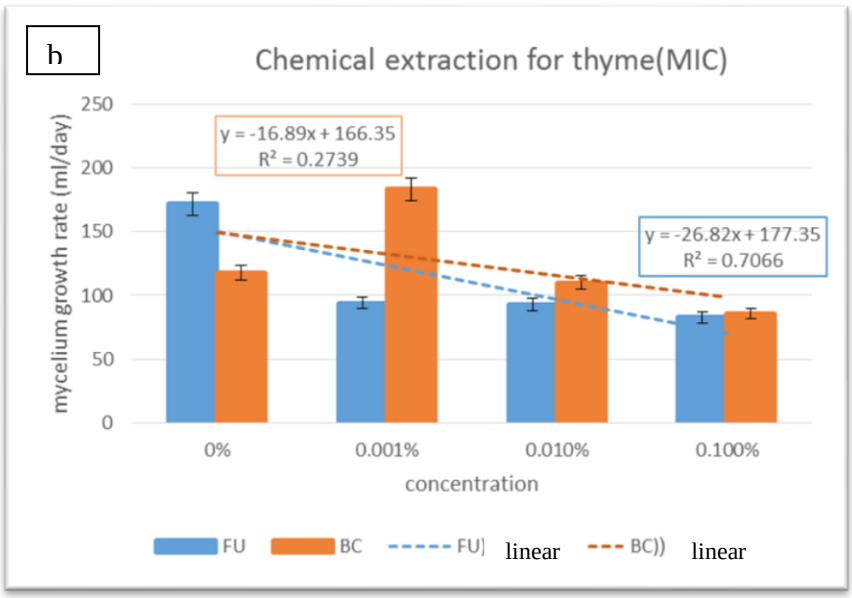


Figure 4.8: Effect of *O. syriacum* leaves extract in (a) different concentrations, (b) minimum inhibition concentration (MIC) on the Mycelium Growth Rate (R) against *B. cinerea* and *F. oxysporum* .

Statistical analyses showed that there are significant differences in the rate of reduction (R) as the concentration of leaves extract increases from .001 to .01, and .1% indicated by small letters (a, b, c,and d) which valued the MIC of *O. syriacum* extracts and EOs as shows in figure 4.9, except at 1%-3% of *O. syriacum* extract and EOs aginst *B. cinerea* were no significant differences in the rate of reduction observed because they are a complete inhibited both fungi growth (R is zero at 1-3%).

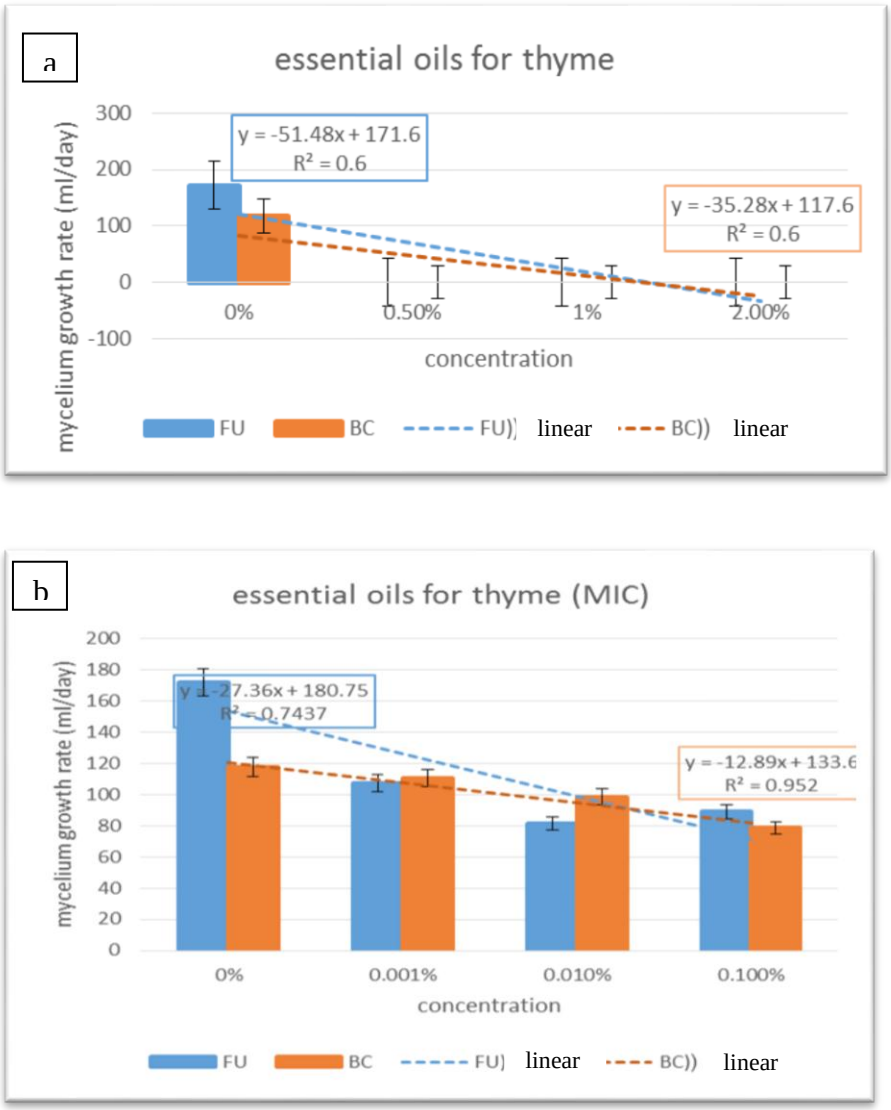


Figure 4.9: Effect of *O. syriacum* EOs in (a) different concentrations, (b) minimum inhibition concentration (MIC) on the Mycelium Growth Rate (R) against *B. cinerea* and *F. oxysporum* .

However, *O. syriacum* hydrosol wasn't significant in the the rate of reduction (R) of *F. oxysporum*, The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference , at the highest concentration used was 3% reduce MGR of *F. oxysporum* by 80% ,and 37% of *B. cinerea* as figure 4.11 shows.

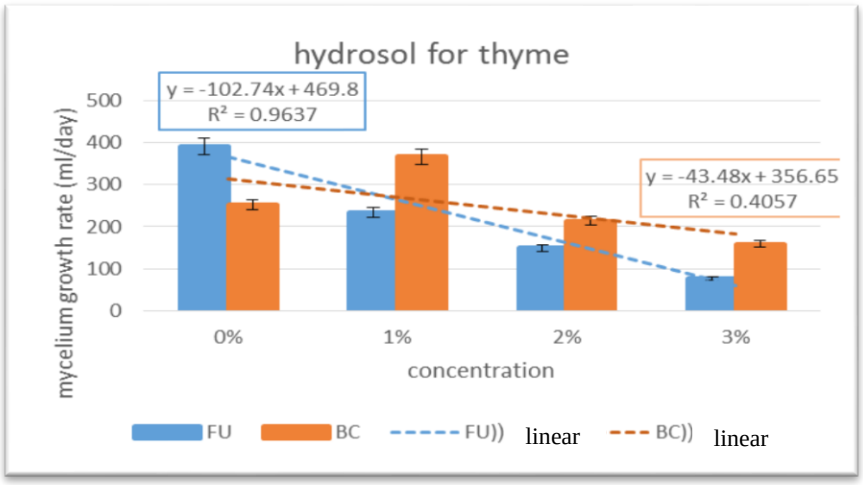


Figure 4.10 : Effect of *O. syriacum* hydrosol in different concentrations on the Mycelium Growth Rate (R) against *B. cinerea* and *F. oxysporum*

4.2 Seed germination result :

To improve Sage and Thyme germination ,many different treatments were done through this experiment, in order to study their effect on seed germination , for achieving rapid, uniform and higher germination which will lead to higher plant stand establishment. And to increase seed germination due to their economic value. so six treatments and control were done, each treatment with 3 replicates, each one contains 40 seed of sage and thyme at 0.3 g seeds (1260 seeds approximately) due to a very smalls seed size, making them very difficult to count. after cultivation on petri dish the reading were taken weekly on the number of germinated seed on each plate in sage and in 1 mm^2 of Thyme plate (1mm^2on the plate contains approximately 70 thyme seed). The four-week replicates average was taken for each treatment, and germination rate were calculated.

4.2.1 Sage, *S .fruticosa* seed treatments :

Comparison of germination percentage in all treatments showed that the highest germination percentage with a numeric value of 80.75% was observed in the kinetin growth regulator for 24 hours , which compared to the control treatment was increased by 9.25% (Figure 4.11). Next higher percentages of germination were obtained for the gibberellic acid treatment for 24 hours with a numerical value of 65% and sand paper scarification treatment with the value of 59%. Germination rates in these treatments have decresed by 6.5% gibberellic acid treatment for 24 hours and12.5% sand paper compared to the control which germination rate was 71.5% (Figure 4.11). In the treatment with chilling the germination percentage was the lowest 22.5% between all treatments, as table 4.3 shows .

Table 4.3: The average of germinated seed, and germination rate during four weeks of *S .fruticosa.*

T/TIME	control	chilling	S. Paper	GA3 /24H	GA3/48H	KIN.24H	KIN.48H
1 Week	0	0	0	0	0	0	0
2week	8.3	0.3	11	9	4.3	12	0
3weeks	22	4.3	20.3	22.3	17.3	25.6	4.6
4weks	28.6	9	23.6	26	21.3	32.3	14.3
GR%	71.5	22.5	59	65	53.25	80.75	35.75

Germination rate(GR%) = n/N*100

where n= total number of germinated seeds during the period,

N= total number of planted seeds

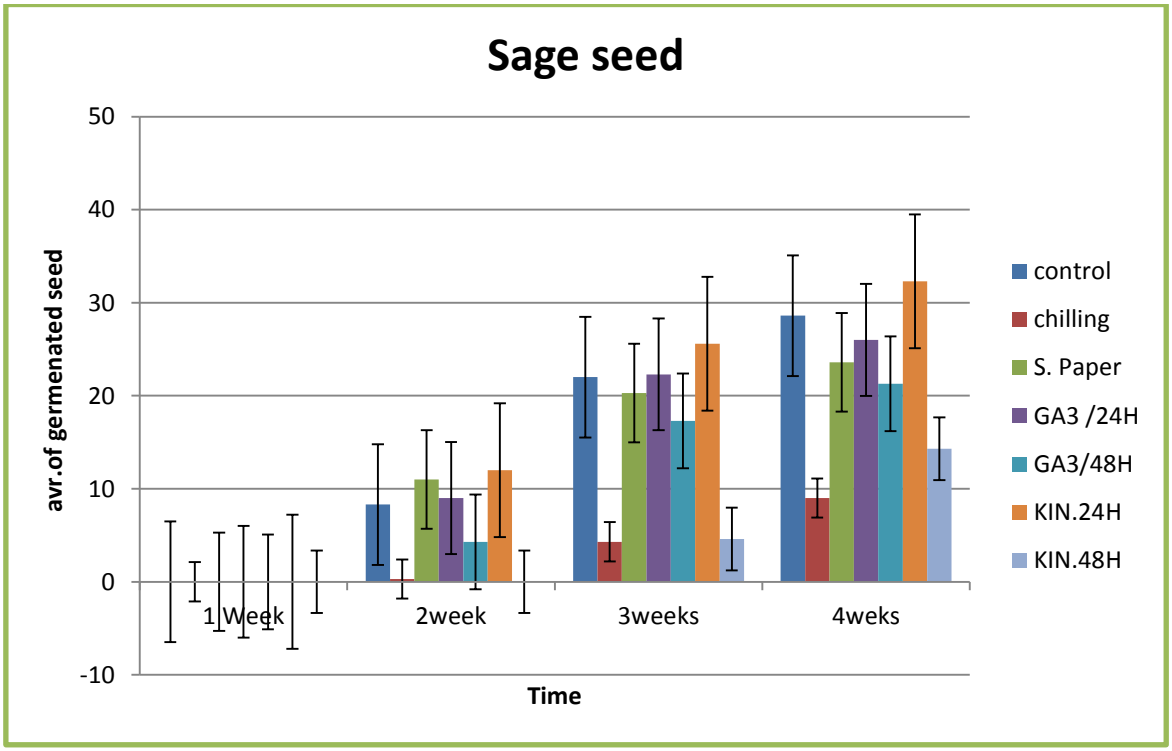


Figure 4.11: Compared *S .fruticosa g*ermination average in all treatments during 4 weeks .

4.2.1.1.Scrape with sandpaper and chilling :

The comparison of germination percentage between the scarification , chilling and control treatment showed that there no significant difference at level of 5% (Table 4.4). Scarification with sandpaper and chilling treatments showed a lower percentage of germination compared to the control treatment. However scarification gave germination average higher than chilling as (Figure 4.12) shows. Germination percentage of seeds scratched with sandpaper was 59%, and chilling 22.5 where in the control treatment this value was 71.5%. After scarification treatment the seed germination decreased by 23.5%

Table 4.4: Analysis of variance to compare of germination rate between scarification, chilling and control treatment on sage seed.

Treatment	Mean	Standard deviation	Standard error
Sand paper	22.780	22.228	9.941
Chilling	7.220	9.290	4.154
Control	26.080	27.759	12.414

Significant differences at 5% level

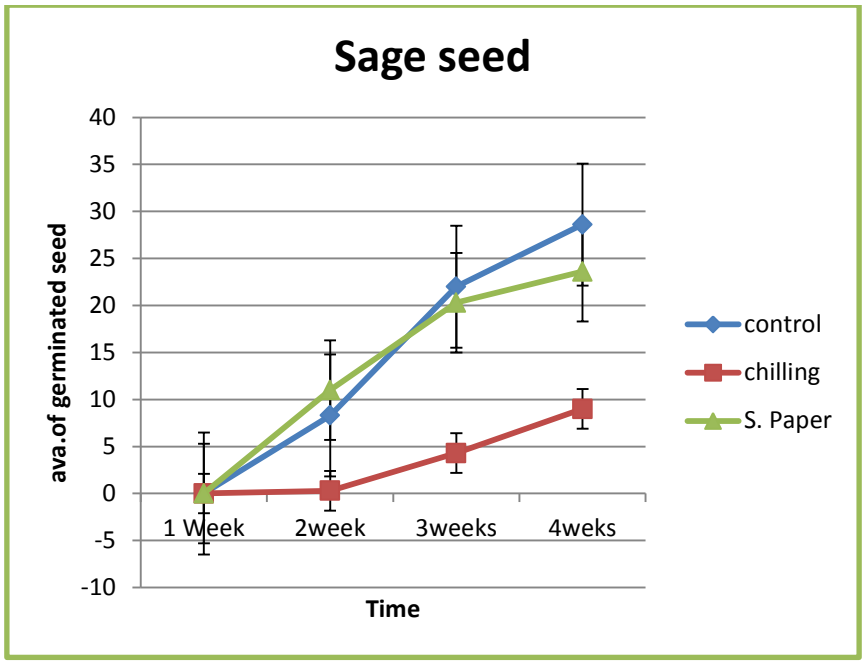


Figure 4.12: Compared of germination rate between scarification, chilling and control treatment on sage seed

4.2.1.2 Gibberellic acid treatments for 24 and 48 hours:

Comparison of the avarge of germination between the treatments with 500 ppm gibberellic acid for 24 and 48 hours, and the control treatment showed no significant difference at at $P<0.050$(Table 4.5). Treatment with 500 ppm gibberellic acid for 24 hours represents the higher germination percentage with a numerical value of 65%, than the germination percentage of 48h treatment with a numeric value of 35.2%. and at both duration of gibberellic acid treatment germination rate was less than control which is 71.5% (Figure 4.13). The results showed that with increasing of the time of treatment with gibberellic acid, germination rate of the seeds decreases.

Table 4.5 : Analysis of variance to compare germination rate of gibberellic acid and control treatment on sage seeds .

Treatment	Mean	Standard deviation	Standard error
GA3/24h	14.325	12.020	6.010
GA3/48h	10.725	10.188	5.094
Control	14.725	12.956	6.478

Significant differences at P<0.050.

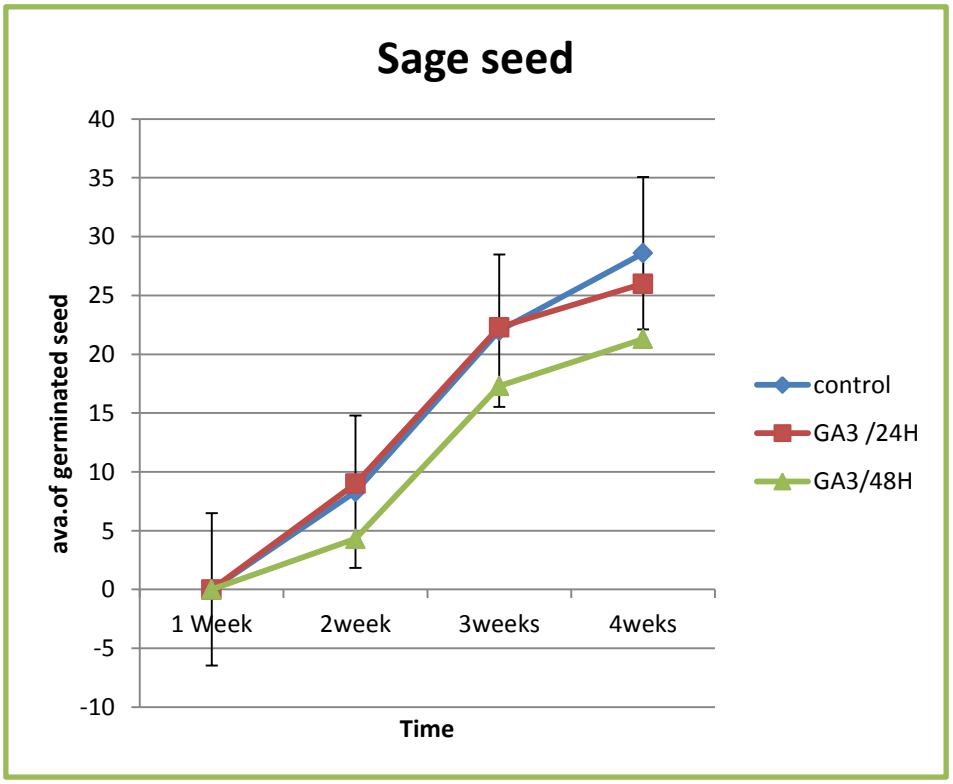


Figure 4.13 : Compared the average of germination between gibberellic acid and control treatmentson sage seed.

4.2.1.3 Kinetin treatments for 24 and 48 hours:

Comparison of percentages of germination between the treatments with 500 ppm kinetin for 24 and 48 hours,and the control treatment showed a significant difference at 5% at 24h treatment (Table 4.6). Treatment with 500 ppm kinetin for 24 hours represents the highest germination percentage with a numerical value of 80.7% (Figure 4.14). The lowest germination percentage related to 500 ppm kinetin treatment was the treatment for 48 hours with a numeric value of 35.7%. There is not a significant difference between the control treatment and treatment with kinetin for 48 hours (Figure 4.14). The results showed that with increasing of the time of treatment with kinetin, germination rate of the seeds decreases.

Table 4.6: Analysis of variance to compare germination rate of kinetin and control treatment on sage seeds

Treatment	Mean	Standard deviation	Standard error
Kin.24h	17.475	14.389	7.195
Kin.48h	4.725	6.742	3.371
Control	14.725	12.956	6.478

Significant differences at 5% level

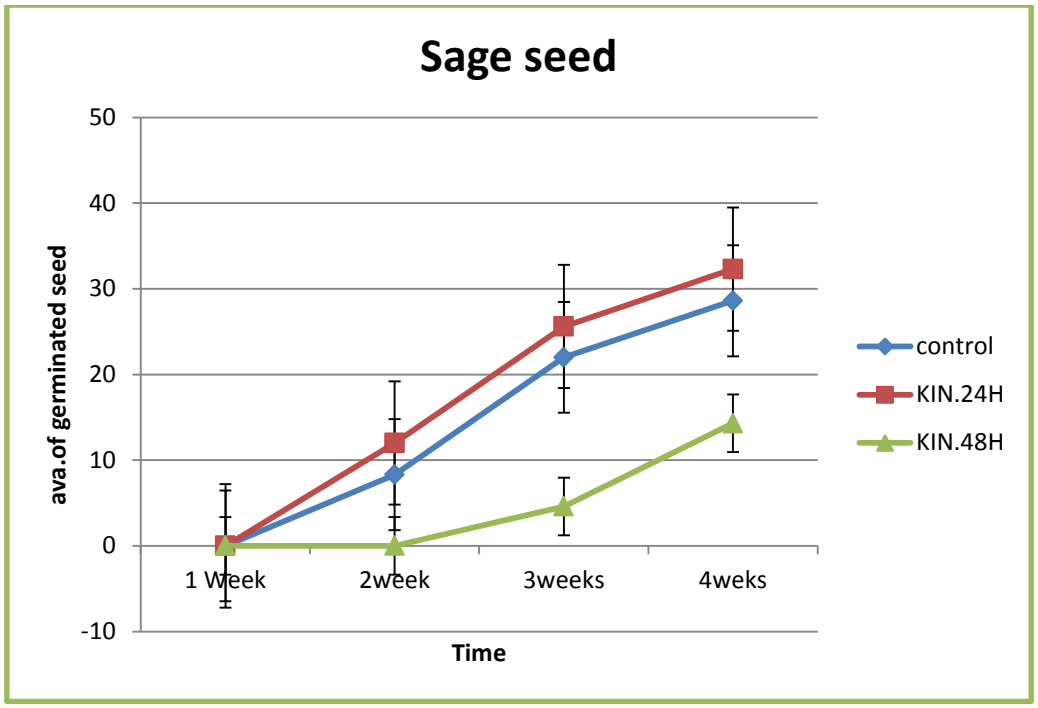


Figure 4.14 : Compared the average of germination between kinetin and control treatmentson sage seed.

4.2.2 Thyme, *O. syriacum* seed tratments :

Comparison of germination percentage in all treatments showed that the highest germination percentage with a numeric value of 91.4% was observed in the kinetin growth regulator for 24 hours , which compared to the control treatment was increased by 45.8% (Figure 4.15). Next higher percentages of germination were obtained for sand paper scarification with a numerical value of 74.7% and gibberellic acid treatment for 24 hours treatment with the value of 55.7%. The increase was 29% and 10%, respectively compared to the control treatment which (45.7%). In the treatments with gibberellic acid for 48 hours (47.6%), and chilling (54.3%) they also increase the germination than control . Results showed that the growth regulator kinetin for 48 hours gave minimum percentage of germination was (17%) which decrease the germination by 28.6% (Figure 4.15), as table 4.7 shows .

Table 4.7: The average of germinated seed ,and germination rate during four weeks of *O. syriacum.*

T/TIME	control	chilling	Sand Paper	GA3 /24H	GA3/48H	KIN.24H	KIN.48H
1 Week	0	0	0	0	0	0	0
2weeks	15.6	13	24	19.3	9	44.3	11.3
3weeks	31	36	52.3	36	29.3	58.3	12
4weks	32	38	52.3	39	33.3	64	12
GR%	45.7	54.3	74.7	55.7	47.6	91.4	17.1

Germination rate(GR%) = n/N*100

where n= total number of germinated seeds during the period,

N= total number of planted seeds

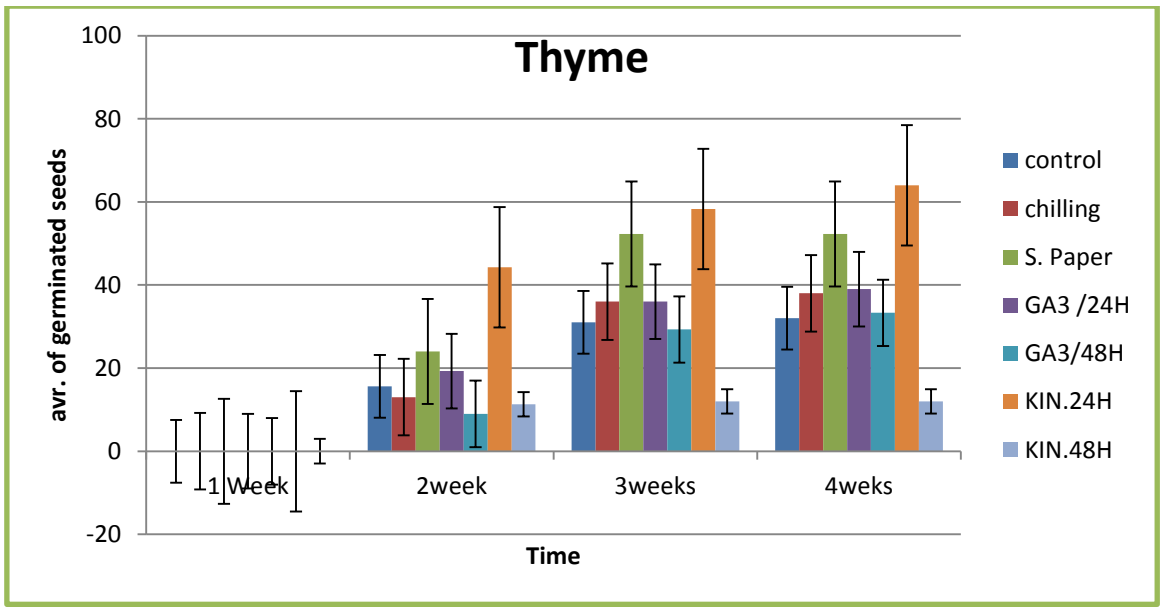


Figure 4.15: Compared *O. syriacum g*ermination average in all treatments during 4 weeks .

4.2.3.1.Scrape with sandpaper and chilling :

The comparison of germination percentage between the scarification , chilling and control treatment showed that there no significant difference at level of 5% (Table 4.8). Scarification with sandpaper and chilling treatments showed a higher percentage of germination compared to the control treatment as (Figure 4.16) shows. Germination percentage of seeds scratched with sandpaper was 74% ,and chilling 54% where in the control treatment this value was 45.7% .After scarification treatment the seed germination increased by 29% and chilling 8%.

Table 4.8: Analysis of variance to compare of germination rate between scarification, chilling and control treatment on Thyme seed.

Treatment	Mean	Standard deviation	Standard error
Sand paper	40.660	28.985	12.962
Chilling	28.260	21.589	9.655
Control	24.860	17.513	7.832

Significant differences at 5% level

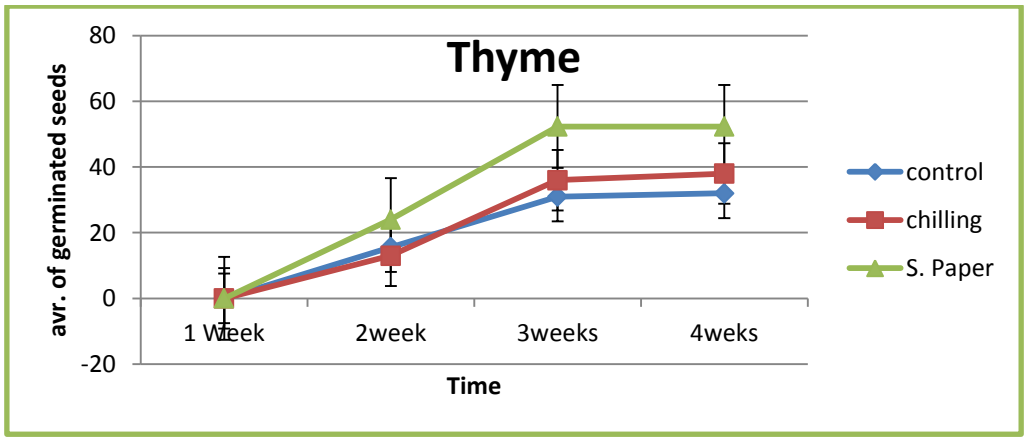


Figure 4.16: Compared of germination rate between scarification, chilling and control treatment on Thyme seed.

4.2.2.2 Gibberellic acid treatments for 24 and 48 hours:

Comparison of the avarge of germination between the treatments with 500 ppm gibberellic acid for 24 and 48 hours, and the control treatment showed no significant difference at at P<0.050(Table 4.9). Treatment with 500 ppm gibberellic acid for 24 hours represents the higher germination percentage with a numerical value of 55.7%, than the germination percentage of 48h treatment with a numeric value of 47.6%. and at both duration of gibberellic acid treatment germination rate was more than control which is 45.7% (Figure 4.17). The results showed that with increasing of the time of treatment with gibberellic acid, germination rate of the seeds decreases.

Table 4.9 : Analysis of variance to compare germination rate of gibberellic acid and control treatment on Thyme seeds .

Treatment	Mean	Standard deviation	Standard error
GA3/24h	23.575	17.948	8.974
GA3/48h	17.900	15.987	7.993
Control	19.650	15.098	7.549

Significant differences at 5% level.

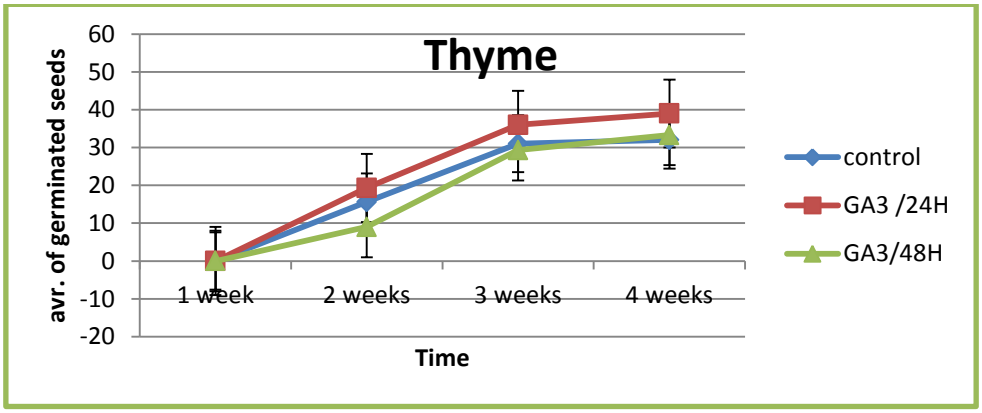


Figure 4.17 : Compared the average of germination between gibberellic acid and control treatmentson Thyme seed.

4.2.2.3 Kinetin treatments for 24 and 48 hours:

Comparison of percentages of germination between the treatments with 500 ppm kinetin for 24 and 48 hours,and the control treatment showed a significant difference at 5% at 24h treatment (Table 4.10). Treatment with 500 ppm kinetin for 24 hours represents the highest germination percentage with a numerical value of 91.7% (Figure 4.18). The lowest germination percentage related to 500 ppm kinetin treatment was the treatment for 48 hours with a numeric value of 17.1%. There is not a significant difference between the control treatment and treatment with kinetin for 48 hours (Figure 4.14). The results showed that with increasing of the time of treatment with kinetin, germination rate of the seeds decreases.

Table 4.10: Analysis of variance to compare germination rate of kinetin and control treatment on Thyme seeds.

Treatment	Mean	Standard deviation	Standard error
Kin.24h	41.650	28.974	14.487
Kin.48h	8.825	5.893	2.946
Control	19.650	15.098	7.549

Significant differences at 5% level

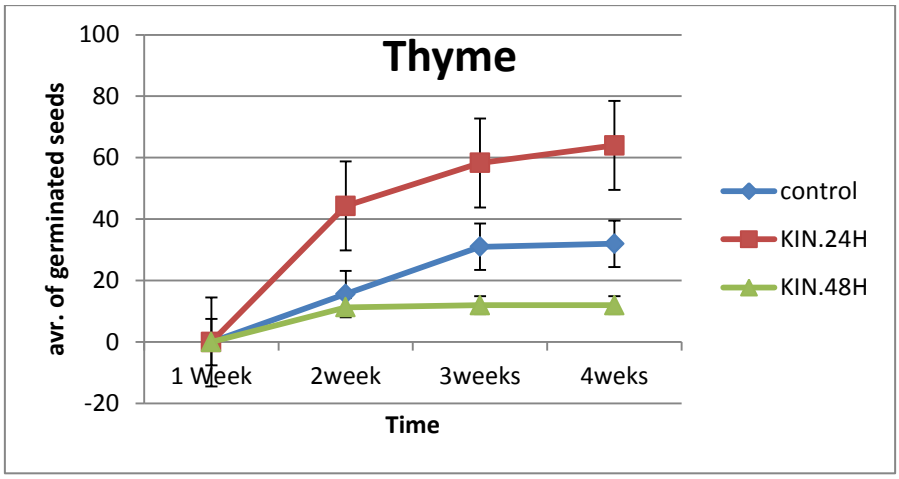


Figure 4.18 : Compared the average of germination between kinetin and control treatmentson Thyme seed.

4.3 Discussion :

4.3.1 Antifungal effects :

The results from experiment have demonstrated that extracts and essential oils from the Salvia were effective to varying extents against the both plant pathogenic fungi. *S.fruticosa* extracts and essential oils showed greater effects against fungi compared to the control .However, it was observed that this effect varied depending on the extracts, essential oils, and hydrosol and their concentrations, and the species of fungus.

The results correspond with Sharawi, et al. 2009 study which showed that olive leaves extracts have antifungal action .Also, results compatible with Yilar, et al.2018 study which showed that the antifungal activity of salvia species leaves extract is concentration-dependent.

Extracts and essential oils from the Salvia species have been shown to be effective at varying degrees against the eight pathogenic fungi in plants. (Yilar et al,2018) S. Offinalis extracts and essential oils exhibited greater effects on fungi compared to controls. However, this effect was observed to vary depending on the extracts, essential oils and fungal species.(Yilar *et al*,2018)

Extracts of *S. fructicosa* produced relatively moderate antifungal activity. At 25% concentration, these extracts showed an incomplete inhibition in mycelial growth being around 75–85% against *Alternaria solani*(Goussous,2010)

According to Šegvić Klarić et al. (2007), the main components of thyme essential oil, which contains p-cymene (36.5%), thymol (33.0%), and 1,8-cineole (11.3%), and pure thymol exhibited antifungal activity. Pure thymol showed an inhibition roughly three times stronger than essential oil of thyme.

Every hydrosols displayed a tendency to be inhibitory. The behavior was related to major chemical constituents of the hydrosol-dissolved essential oils (Inouye *et a1*.,2009). several researches concluded that the effects of hydrosols were concentration and time dependent.(Boyraz & Ozcan,2005) that's why our hydrosol results were not significant effect both pathogenic fungi, it should be with high concentration reach 15%. Of the 16 Turkish spice hydrosols, nise hydrosols, cumin, fennel, mint, pickling herbs, oregano, savory and thyme showed strong activity inhibiting the growth of *Aspergillus parasiticus* (aflatoxin forming mold). Sumac, seafennel, rosemary, sage, aegean sage, laurel, and basil hydrosols failed to inhibit mycelial fungal growth (Ozcan,2005). However three Australian lavender species (Lauandula spp.) did not show any antifungal activity against four tested fungi, while their essential oils were involved (Moon et a1.2007).

As mentioned earlier, the composition of essential oils can vary according to climate (temperature, light, and humidity), soil composition, geographical location and vegetation stage. In addition to the antifungal properties of essential oil may be related to the presence of high concentrations of major compounds , or to a synergistic effect with some active components ; This explains the high effectiveness of the oil based on the results of research of the oil that inhibiting the growth of both fungi at low concentrations (0.5, 1,2%)of Sage and Thyme , compared to the efficacy of hydrosol on concentrations (1,2,3%)., So to get the optimum efficacy of Hydrosol, high concentration more than 10% should be used, which must be studied later.

Sage was found less potential compared to Thyme; Thyme showed high inhibitory effect, even for the very low concentration of leafs extracts and essential oils against both pathogen tested. The obtained results showed that the inhibitory effect was dependent on the amount of the EO employed because thyme EOs rich in thymol and carvacrol that could control a wide range of fungi. The results compatible with Azizi *et al*. (2008) study which observed that radial growth of *P. italicum* was completely inhibited by thyme (500 mg/L), *Saturega hortensis* and *Thrachryspermum copticum* (1000 mg/L). Moreover, Abdolahi et al. (2010) reported that thyme essential oils exhibited strong antifungal activity against *Botrytis cinerea* and *Mucor piriformis*

4.3.2. Seed germination

Shakeri-Almoshiri et al. (2009) illustrated the sandpaper's positive impact in promoting seed germination of *Teucrium polium L*. Scarification *O. syriacum* with sandpaper caused thinning hull of seed and thus reduced mechanical strength from the bud. Being seed germination successful of confirms the effect of mechanical strength hull against out of bud. In addition to who Hejabi & Soltanipoor (2006) have shown that pre-eruptive

mechanical scarification can influence *Salvia mirzayanii's* germination characteristics to increase the germination of this medicinal plant.

Cooling treatment at 5 ° C improved germination of the *Ferula gomussa* for 2 weeks (Macchai et.al., 2001). Khakpoor *et al*.(2015) results showed that the 2 and 4 weeks chilling treatment significantly reduced the percentage of germination of *S. verticillata* L. Reduced germination at low temperatures may be due to the negative effect of low temperatures on enzyme activity, thus reducing the metabolic and biosynthetic activity needed for germination, growth and development of the seeds.so chilling treatment in our experiment didn't give significant effect on both plant according to previous studies cooling should be in sufficient time more than two weeks not in 2 days.

In this experiment , the germination rate of gibberellic acid seeds treated for 24 h were significantly increased germination compared to the control treatment.These results are in line with the findings of Fulbright et al. (1983), which stated that gibberellic acid stimulates the germination of seeds.Also Kochacki & Azizi (2005) found that 500 ppm gibberellic acid treatment had the most positive effect to promote sprouting of *T. Polium* .

Cytokinins, including kinetin, are naturally produced in tissues and organs that develop or meristematically, including the root tips (Rademacher, 2015). Christina *et al*.(2019) explained that plants growth regulator (PGRs) with the greatest overall effect on seed germination and plant growth. Their potential benefits, such as breaking seed dormancy, improving early seedling emergence and improving shoot length, are expected to provide a competitive advantage for native plants over aggressive non-native species.

Conclusion :

The *S.fruticosa* and *O. syriacum* leaves extracts and essential oils have high antifungal activity against *B. cinerea* and *F. oxysporum* in vitro . The reduction of mycelial growth rate was positively correlated with increasing the leaves extract or EOs concentrations, with the level of effectiveness varying depending on dose, extract, and plant pathogen. the results of the present study and other studies investigating alternative antifungal agents are of particular importance for managing pathogenic fungi ,so it's very important to increase the cultivated areas of sage and thyme , in order to efficiently use them to produce bio-pesticides. Based on the results of seed germination treatments , it is better to treat seeds with Cytokinins (Kinetin) to increase seed growth and increase the production of leaves of these plants for the production of bio pesticides .

Recommendations:

Referring to this work, the following recommendations for future work would be outlined:

- In vivo studies are still needed to determine the effect of both plants extracts and EOs on whole plants in greenhouses and field .
- Determine the formula of bio- fungicides for each plant extract or EOs and to identify the individual active compounds .
- Determine the mechanism of leaves extract and EOs to act as antifungal
- Further chemical analysis using GC-MS to specifically determine the bio active compound in these nature products.

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Abstract in Arabic :

دراسة الفعالية الحيوية ضد مسببات الأمراض الفطرية النباتية لنباتين طبيين (المرمية و الزعتر) ودراسة إنباتهم

إعداد : فاطمة الزهراء حسام الدين مناصرة

بإشراف: الدكتور خالد صوالحة.

الملخص:

يعد فطر البوترايتوس المسبب الرئيسي لمرض العفن الرمادي ، وهو احد اكثر الامراض الفطرية المدمرة للنباتات حيث انه يصيب اكثر من 230 موع نبات مختلف مثل البندورة والعنب و الفراولة وغيرها ، مسببا عدة أعراض تؤدي الى خسائر اقتصادية في المحاصيل. اضافة الى فطر الفيوزاريوم الذي يعيش في التربة مسببا عدة امراض مثل امراض الذبول والتعفن للسيقان والابصال على اهم المحاصيل الاقتصادية مثل البندورة ، بطاطا ، بقوليات و القثائيات والموز .كلى الفطرين يمكن السيطرة عليهم ومحاولة مكافحتهم بعدة طرق مثل بعض الممارسات الزراعية كإزالة الاوراق المصابة و استخدام الري بالتنقيط والمحافظة على نظافة الادوات وغيرها الكثير من الممارسات الزراعية . بهدف تقليل وجود الفطر وانتشاره، اضافة الى استخدام الطرق الكيميائية من مبيدات كيميائية مختلفة للقضاء عليها والوقاية منها ، لكن ولسوء الحظ قامت هذه الانواع من الفطر بتطوير مناعة ومقاومة عالية ضد هذه الكيماويات التي بدورها لها أثار سلبية على البيئة و صحة الانسان والحيوان ، لهذا بدأ البحث عن بدائل للسيطرة على هذين الفطرين . من ضمن هذه البدائل كانت المكافحة الحيوية والتي تشمل المستخلصات النباتية . في العقود الاخيرة تم التوجه للنباتات الطبية من ضمنها (المرمية والزعتر) كمصدر مهم لمركبات فعالة تستخدم في مجالات عدة منها الطبية والتجميلية وصناعات الاغذية .وذلك يعود لأن هذه المواد تعتبر أمنة على الصحة ،اضافة الى ان الدراسات اثبتت الفعالية الحيوية لمستخلصاتها وزيوتها العطرية كمضادات فطرية وبكتيرية ومضادات اكسدة لذا كان الهدف من هذه الدراسة ايجاد بديل للمبيدات الكيميائية المستخدمة بكثافة في فلسطين بمبيد عضوي طبيعي أمن وصديق للبيئة من مستخلص و الزيت والمياه العطرية لكل من الميرمية والزعتر من خلال فحص المضادات الفطرية الناتجة من هذه النباتات ضد كل من فطر البوترايتوس و الفيوزاريوم . تم جمع أوراق الزعتر والمرمية و تجفيفها وطحنها لاستخلاص أوراقها باستخدام ايثانول 99% وعزل زيوتها العطرية بطريقة التقطير بالبخار ومن خلالها تم استخراج المياه العطرية أيضا . ولدراسة التأثير البيولوجي لهذه المستخلصات تم استخدام فحص يقيس معدل نمو الغزل الفطري الذي ينمو على الوسط الغذائي (Media) التي تحتوي على عدة تراكيز لكل مستخلص من النباتين .

أظهرت النتائج أن كلا النباتين لها تأثير على تقليل نمو الفطرين ، بحيث أن معدل نمو الفطرين يقل كلما زاد تركيز كل من المستخلص والزيوت والمياه العطرية مقارنة مع العينة التي لم يضف عليها أي مستخلص من النباتات عليها (control) حيث أن نتائج عينات الميرمية أظهرت أن بعد اضافة كل من المستخلصات على حدى فإنها اظهرت أثر على نمو الغزل الفطري لكلا

الفطرين ،حيث أنه بعد التحليل الاحصائي ظهر أن هناك اختلاف معياري في معدل انخفاض نمو الفطرين كلما زاد تركيز مستخلص اوراق المرمية وزيتها العطري حتى يصل الى التثبيط الكامل 100%، في حين ان مياها العطرية لم تظهر اختلاف معياري بين التراكيز المستخدمة في البحث. في المقابل اظهرت نتائج نبات الزعتر أعلى نسبة تثبيط نمو الفطرين 100% عند معاملتها بالمستخلص والزيت العطري لأوراق الزعتر وتبين أن اقل تركيز يمكن للفطرين ان ينموا عليه هو (MIC) 0.1%في حين أن مياهه العطرية لم تظهر بعد التحليل الاحصائي أي اختلاف معياري في معدل انخفاض نمو الغزل الفطري .

بعد ظهور نتائج الفعالية البيولوجية لكلا النباتين وتأكيد فعاليتهم ضد هذين الفطرين ولأهمية زيادة المساحات المزروعة بهذه النباتات لاستغلالها الأمثل في انتاج المبيدات الحيوية نظرا لقيمتها الاقتصادية المرتفعة ولتحسين نموها تم اجراء عدة معاملات على بذورها لمعرفة أثر هذه المعاملات على نسبة انبات البذور (Germination) وذلك للحصول على اسرع وأعلى نسبة انبات متماثله من خلال ست معاملات مختلفة (الخدش بورق الزجاج، التبريد ، ومعاملتها بهرمونات نباتيه الجبرالين ، الكاينتين بتركيز 500ppm على فترتين كانت 24 و 48 ساعة) ومقارنة نتائج هذه المعاملات بالبذور غير المعاملة (control) .بحيث اظهرت النتائج لهذه المعاملات اعلى نسبة انبات لبذور الميرمية كانت بعد معاملتها بمنزم النمو الكاينتين (kinetin) لمدة 24 ساعة 80.7% في حين أعطت المعاملة بالتبريد أقل نسبة انبات بنسبة 22.5% بين جميع المعاملات .كما اظهرت نتائج المعاملات لبذور الزعتر اعلى نسبة انبات بعد معاملتها الكاينتين (kinetin) لمدة 24 ساعة90.1% مقارنة بالبذور غير المعاملة بمعدل زيادة 45.8% ثم تأتي معاملة الخدش بورق الزجاج 74.7% في حين كانت أقل نسبة انبات تحت معاملة الكاينتين (kinetin) لمدة48 ساعة بنسبة انبات 17%.في نهاية جميع نتائج الدراسة تبين ان لكل من مستخلص وزيوت ومياه العطرية لكل من المرمية والزعتر خصائص مضادة للفطريات(البوترايتوس والفيوزاريوم)لذلك يمكن الاستفادة منها في انتاج مبيدات فطرية حيوية لتقليل الامراض النباتية مسببها الرئيسي احدى هذه الفطريات وايجاد أحسن تركيبة كيميائية لمبيد فطري عضوي يحتوي هذ المواد الفعالة لهذه النباتات .