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Use of Biopesticides from wild flora Palestine against crops pathogens

Hadeel Faried Abd Al-Haleem Irfaeya

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Use of Biopesticides from wild flora Palestine against crops pathogens

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Dedication

To whom was the best help to me on this journey, to the lasting bond and shoulder, to who planted the seed of love for learning and knowledge in us, and to whom fights the universe on our way, to my dear parents "Faried and Sara".

To my beloved husband "Mohammad", who always brought light to my darkness, the source of inspiration for every step of my study, constant provision, emotional support, and strength when I gave up, the good example of hard work.

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To all my supportive friends during this journey,

First and foremost, to my homeland Palestine, the land of creativity and inspiration,

I dedicate this research.

Hadeel Faried Abd Al-Haleem Irfaeya...

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: 

Hadeel Faried Abd Al-Haleem Irfaeya

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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 cause many economic losses. Also, *F. oxysporum* a soil-borne facultative pathogen with 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 in several ways, such as cultural and chemical controls. Unfortunately, they develop high resistance to several chemical fungicides which have negative environmental, plant, animal, and human impacts. So several alternatives were suggested including biological control and plant extracts to reduce them. In recent decade's medicinal plants include *S. dominica L., Varthemia iphionoides Boiss., Achillea fragrantissima (Forssk.) Sch. Bip* and *Thymus persicus Jalas*. F.Rech ex Ronniger arising used as a potential source of significant compounds in medicines, cosmetics, and food industry. Because of the safety concerns it contains, and its bioactive essential oils which were proved by several studies that have antifungal and antioxidant effects. Thus the objective of this study is to investigate the antifungal properties of these medicinal plants against *B. cinerea. F. oxysporum. S. dominica, V. iphionoide, A. fragrantissima, and T. persicus* leaves were collected in 2019 from different locations in bani Na'im. Then leaves were extracted by using 99.0% ethanol. HPLC analysis with PDA detector was performed for mixture of standards and plant extracts. Mixture of 17 standards of polyphenolic compounds and flavonoids were separated using an RP-HPLC method. The HPLC analysis of the ethanolic extract for *Salvia dominica* showed the presence of 3,4-dihydroxybenzoic acid, kapmpferol, chlorogenic acid, quercetin, syringic acid, rutin, and verbascoside. On the other hand, the ethanolic extract of *Achillea fragrantissima* showed the following compounds: rutin, chlorogenic acid, kampferol, quercetin, trans-cinnamic acid, sinapic acid, and p-coumaric acid. *Thymus persicus extract* analysed using RP-HPLC method revealed the following compounds: rutin, quercetin and kampferol, while HPLC chromatogram of *varthemia iphionoide* extract showed rutin, chlorogenic acid and kampferol identified in this extract..The effect of each extract against *B.*

cinerea and *F. oxysporum were* tested by using mycelium growth rate assay using potatoes dextrose agar (PDA) as fungal media. Results showed that all of these plants leaves extracts had high antifungal effect on mycelium growth against both pathogenic fungi compared to the negative control sample (without extract). Results showed that *S. dominica, V. iphionoide, and A. fragrantissima, T. persicus leaves* extracts have a high antifungal effect on mycelia growth of *B. cinerea* and *F. oxysporum* pathogens. Statistical analyses showed significant differences in the mycelium growth rate of fungus as the concentration of leaves extract increases. *T. persicus* leaves extracts showed strong antifungal activity that inhibit the mycelium growth rate (R) and reached up to 100% against both pathogenic fungi (*Botrytis cinerea* and *Fusarium oxysporum)*. This study concludes an important potential of some selected medicinal plant extracts in controlling *B. cinerea* and *F. oxysporum* especially when combined with a reduced dose of chemical fungicidal compounds which gives this control method an integrated approach dimension that can aid in reducing the environmental and health hazards of potential fungicides. However, further studies in the field and on the mode of action and stable formulation of these medicinal plant extracts are needed before reaching the stage of wide-scale application and conclusions in the field of gray mold disease control.

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GC/MS	Gas chromatography–mass spectrometry
S.dominica	*Salvia Dominica*
V.iphionoide	*Varthemia Iphionoide*
A.fragrantissima	*Achillea Fragrantissima*
T.persicus	*Thymus persicus*
MGR	Mycelial Growth rate
PDA	Potato Dextrose Agar
HPLC	High performance liquid chromatography
BCAs	Biological control agents
FW	Fusarium Wilt
RP-HPLC	Reverse Phase High performance liquid chromatography
WV	Wavelength

Chapter one: Introduction

1.1 Natural products:

In ancient times, plants were used in the treatment of diseases, so that folk medicine is considered one of the usual and important treatments in developing countries for historical and cultural reasons, so there must be a scientific evaluation of medicinal plants and also the validity of their use must be verified and studied scientifically and their health, harmful and dangerous effects.

700 species of plants are famous for their medicinal value in the Middle East, and their medicinal importance is one of the main sources of biologically active substances. Records have provided that 80% of people use herbs as a primary health care system. 50% of prescription products from Europe and the USA are natural products. (Rasooli, 2011)

1.2 *Salvia dominica* (Khowwekha):

Salvia belongs to the *Lamiacea*e family (formerly known as Labiatae). Plants belonging to the lamiaceae family are known for their anti-inflammatory, cytotoxic and antiviral properties. They are rich in diterpenoids, triterpenoids, sesterterpenoids, phenolic compounds and other compounds, Salvia distinguishes being the only genus in the lamiaceae family that produces sesterpenes. Salvia plants have medicinal and culinary properties. Salvia plants recorded that include 25 species, including *Salvia dominica*. (Al-Eisawi, 2013).

Salvia dominica belongs to the biological-geographical areas of the mediterranean region and Iran. *Salvia dominica* is a perennial herbaceous shrub with a woody base (30-70 cm), a large number of basal branches and aromatic, heavily hairy leaves with a length of (3-9 cm). The flowers are simple, cream in color and have a yellow lower lip as shown in Figure 1.1. The length of the flowers ranges from (1.5-2 cm). It flowers in the spring (April-June). The salvia plant is considered an anti-colic astringent for the stomach and intestines, so it’s used to treat colds, stomach aches and digestive problems (Abdallah et al., 2013).

Salvia plants are important for the cosmetic, pharmaceutical and food industries. Some salvia species are prescribed for various ailments, including bronchitis, tuberculosis, bleeding, and menstrual cramps. They have many antimicrobial, anti-inflammatory, anti-tumor, antifungal and anti-diabetic properties. Because of this importance, salvia have been the subject of chemical, phyto-pharmacological and ethno-pharmacological studies (Wu et al., 2012).

Figure 1.1: *Salvia dominica* plant (Khowwekha).

Essential oil of *Salvia dominica*:

GC/MS analysis was performed for dry and fresh essential oils of *Salvia dominica* and the results showed that monoterpenes (87.8% and 72.4%, respectively). Oxygenated monoterpenes, mainly alcohols, dominated. Linalool (alcoholic monoterpenoid (31.4% and 18.3%, respectively)) was found to be the major oil in the fresh and dry plant oils, followed by alpha-terpin (alcoholic monoterpenoid (31.4% and 18.3%, respectively.)), followed by alpha-terpineol (25.4% and 15%, respectively). 50% of the volatile molecules in the vegetable oil of the fresh plant are accounted for by these two compounds. The presence of a high percentage of linalool imparts a strong aromatic odour to the fresh buds. The presence of dihydrocarvacrol and thymol was detected at concentrations of 10.7% and 7.5%, respectively, in the essential oil produced by drying Salvia dominica in the presence of linalool and alpha-terpineol. Thymol was not present in the fresh oil. (Abdallah et al., 2013).

1.3 *Varthemia iphionoide* (Shtaila):

Varthemia iphionoides is a dense perennial herb with a height of (30-80 cm), consisting of several branched, sticky, aromatic stems. It is the most widespread species of *varthemia ssp*. While there are other species such as *Varthemia candicans* and *Varthemia montanas*, *varthemia iphionoides* is characterized by yellow flowers as shown in figure (1.2). (Afifi et al, 2004)

The most common distribution areas of *Varthemia iphionoides* are the rocky habitats of the mediterranean region and iran. There are rumors that the jordanian people use the medicinal extract of *Varthemia iphionoides* as a medicine to treat indigestion and diabetes. (Abdallah et al. 2013, Oran et al. 1998).

Figure 1.2: *Varthemia iphionoide* (shtaila) plant.

The flavonoid compound is one of the main compounds of the essential oil of the plant *Varthemia opium*, which has been isolated to discover its importance and discover its action as an antifungal (Sharawi et al, 2009).

1.4 *Achillea fragrantissima* (Qaisoom) :

The *asteraceae* family includes more than 100 species found in different parts of the world, including the plant *Achillea fragrantissima*, whose biodiversity in the wild is threatened by habitat destruction, overexploitation and threats from agricultural practices, climatic changes, and also social and cultural changes (Al-Esawi, 1998).

The preservation of this plant and the idea of sustainable use of its genetic resources to meet the demand of future needs. Because of its importance. It is chiefly used in the treatment of diabetes, intestinal colic, to lower cholesterol, and as a carminative, in dysmenorrhea and various infections. Bedouins and rural people in Jordan use this species to treat stomach pain and diabetic diseases. It can also be used for inflammation, nosebleeds, excessive menstruation and hemorrhoids. It is very important to identify and prioritize threats to biodiversity by identifying areas for monitoring, management, and protection. (Moritz et al, 1998).

The plant *Achillea fragrantissima* is a medicinal shrub (see Figure 1.3) with a beautiful aromatic diploid odor as it contains this number of chromosomes: 2n = 18.

Figure 1.3: *Achillea fragrantissima* (Qaisoom) plant.

The essential oils *of Achillea fragrantissima* are a mixture of volatile compounds, which are mainly terpenoids, baside mono- and sesquiterpene lactones, azulene and some other compounds. (Barel et al., 1991)

1.5 Thymus persicus (Zaatar*) :*

The genus *thymus L. (Lamiaceae)* consists of 300 species of perennial herbs distributed throughout the world. The thymus species is important because it is a natural source of phenolic monoterpenes and oleoresin oils. Thyme has been used in traditional medicine for centuries for its antiseptic, repellent, antimicrobial, antiviral, and antiviral properties. An interesting constituent of the thyme plant is the pentacyclic triterpenoids (PTs), i.e. betulinic acid (BA), oleanolic acid (OA) and ursolic acid (UA). The value of these compounds is very high due to their biological properties as they have anti-inflammatory, liver protective and antineoplastic anti-HIV, antimicrobial, antifungal, anti-ulcer, anti-infectious prophylactic against hypoglycemia, anti-hyperlipidemia thymus (Liu et al. 2012, Chudzik et al. 2015).

Persian thyme with the scientific name Thymus persicus Jalas. F.Rech ex Ronniger has different nutritional uses. It is fermented, cooked and used in Iranian medicine. It is anti-flatulent, anti-inflammatory, digestive, and expectorant. Thyme also has antibacterial, antifungal, antiviral, antioxidant and aromatic properties. Thyme is carminative. For insects.. (Asghari et al., 2019).

Figure 1.4: *Thymus persicus* plant (Zaatar)

The major components of the 25 identified compounds of T. persicus are carvacrol (39.0% and 27.1%), geraniol (15.7% and 9.4%), pecamine (7.5% and 10.2%), thymol (6.5% and 11.9%), y -terpinene (6.1% and 6.5%) and geranyl acetate (5.3% and 5.3%) before flowering and at full flowering stage, respectively.

1.6 Fungal background:

1.6.1 *Botrytis cinerea*:

The *Botrytis* fungus belongs to the kingdom E*umycota,* the tribe *Ascomycota,* the class *Leotiomycetes,* the order *Helotiales,* and the family S*clerotiniaceae*. The name *Botrytis cinerea* goes back to von Haller (1771) in his "Synopsis Methodica Fungorum", published in Zurich, Switzerland. *Botrytis cinerea Pers.* most important Botrytis of 22 species. A pathogen that can be very destructive in some crops found all over the world, damaging any plant or part of a plant. Sometimes it is characterized by early latent infections that do not damage the fruit until ripening.(Rosslenbroich and Stuebler, 2000).

1.6.1.1 The host range and importance of *Botrytis cinerea*:

Botrytis cinerea Pers. is a necrotrophic plant pathogen that causes severe pre- and postharvest losses in more than 200 crop species around the world. It causes soft rot of all aboveground plant sections as well as postharvest rot of vegetables, fruits, and flowers, and produces the characteristic gray conidiophores and (macro) conidia of these diseases.

The fungal pathogen *Botrytis cinerea* is one of the most important fungal plant pathogens. It is not only a model plant pathogen but infects over 1400 different hosts out of 586. Its success as a pathogen is due to its wide host range, rapid adaptation to fungicides, infection of multiple life stages of the host, and numerous infection and overwintering methods.

Currently, infections by *B. cinerea* and the closely biologically related fungi monilinia and sclerotinia on grapes, vegetables, berries, and stone fruit are of considerable economic importance worldwide. The economic importance is even greater when one considers that these crops are not only at risk in the field but also during transport and storage of the ripe fruit.

Most damage occurs in mature or senescent tissue on dicotyledonous hosts. Unlike many other plant pathogens, an extremely wide range of plants can be attacked by single necrotrophic fungal isolates. *B. cinerea* damages various plants, including potatoes, cereals, and ornamentals, including fruits in various parts, as well as certain monocotyledonous hosts. It also damages horticultural plants grown in greenhouses prior to harvest, and even the seedling stage of many host plants. These include broccoli, beans, cabbage, carrots, lettuce, sweet potatoes, kiwis, chickpeas, and other protein legumes. As well as roses and gerberas. Sunflowers can also be contaminated, which are an important oil crop. (Breeze, E. 2019).

1.6.1.2 *Botrytis cinerea* symptoms:

B. cinerea causes a wide range of symptoms (Fig. 1.5), which are difficult to generalize to all plant organs and tissues. The most common symptoms on leaves and berry fruits include soft rot, which is accompanied by parenchyma tissue collapse and soaking, as well as the quick formation of grey masses of conidia (Fig. 1.5a,b). The dark water-soaking sensation does not appear until after cutting on thick-skinned fruits like kiwifruit. In many fruits and vegetables, such as courgettes (zucchini), cucumbers, snap beans, strawberries, and apples, infection originates on the connected senescent blossoms and then spreads as soft rot to impact the surrounding developing fruit (blossom end rot). Symptoms on flower petals range from minor 'pock' marks to full-blown soft rot, depending on the environment (Fig. 1.5c). On greenhouse-grown tomatoes, the fungus causes the most harm to stems at pruning wounds, where it can consume the entire stem. After harvest, soft rot on mature tomato fruit is common. The symptom 'ghost spot' on immature tomatoes has been linked to successful host defense, although it makes the fruit unmarketable. The disease targets mature to ripening leaves, causing a wedge-shaped maroon lesion with yellow borders that spreads to the nodes on the vegetative stems (primocanes), as well as a visible pale brown, rapidly spreading lesion (up to 15 cm) in the primocane bark of the stem. Because of the periderm layers, this infection does not enter the axillary buds, but it does delay the growth of buds at infected nodes, preventing them from producing fertile lateral shoots the next year. Raspberry stem lesions turn white after winter dormancy and reveal enormous black sclerotia, which produce masses of grey conidia in the spring. Asymptomatic *B. cinerea*

infection of flower styles (detected by fluorescence microscopy) causes early death of developing fruit related with ethylene production in blackcurrants, a disease known as 'run-off.' (Williamson et al., 2007).

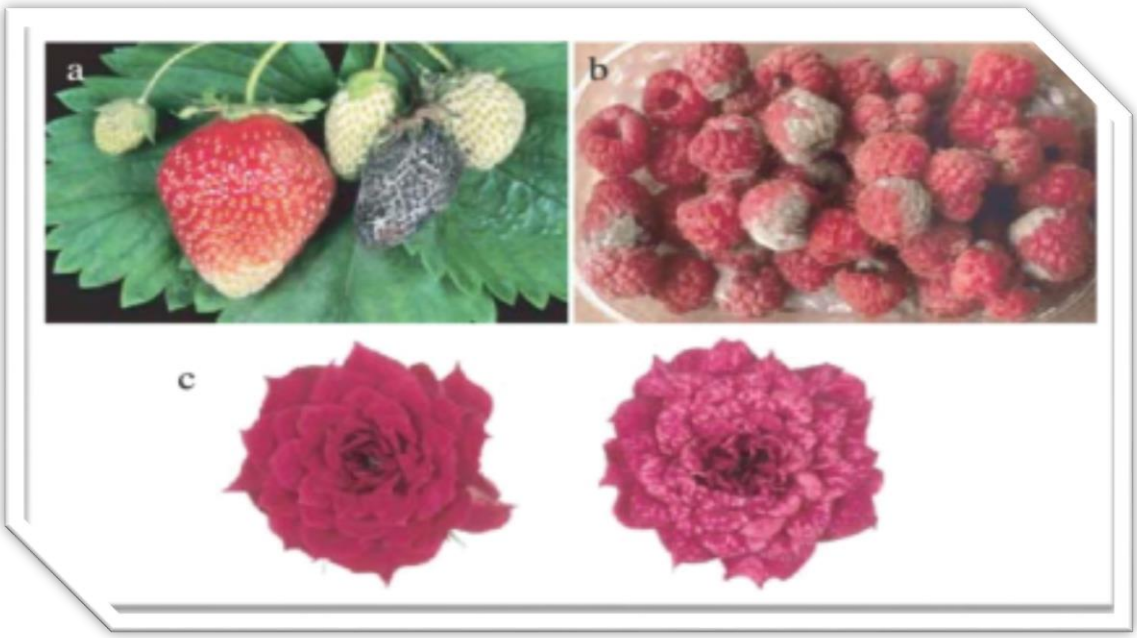


Figure 1.5: *Botrytis cinerea* infection symptoms. (a) Strawberry fruit with a grey mold. (b) Raspberry fruit with a grey mold. (c) Rose petals with lesions (right) after inoculation with dry conidia and 48 hours of incubation at 100% RH, compared to non-inoculated control (left). Reprinted with permission from Williamson et al (1995).

1.6.1.3 *Botrytis cinerea* life cycle

B. cinerea's mycelium is branching, septate, and hyaline to brown in color. Conidiophores are tall, thin, irregularly branching in the terminal half, with expanded or rounded apical cells, bearing concurrent clusters of conidia on short denticles, growing directly from the mycelium or sclerotia. Smooth, hyaline, or gray egg-shaped conidia with a mean length of 10m and a mean width of 5m. Sclerotia, or survival structures, are frequently found. Figure 1.6 represents the life cycle of a fungus in both sexual and asexual stages. Sclerotia is formed by the fusion of fungal branches into a spherical mass that is initially hyaline but later turns brown or black due to the deposition of melanin pigments in the outer cortex.

Over time, the sclerotia is protected from desiccation, UV rays, and microbial attack by the pigmented envelope and β-glucans surrounding the mycelium. Sclerotia germinate after a sexual process, when environmental conditions are optimal for the fungus, by elongation of the apothecium or by the release of conidiophores. On the other hand, the development of conidiophores is the most common method of germination. Chlamydial spores are short-term structures that help the fungus to overcome short unfavorable periods on plant surfaces. They are generated by the transformation of the vegetative parts of the mycelium and are released by the decay of the hyphae.

Significant amounts of microconidia are observed in all the crosses that make up the sexual bodies during the sexual reproductive cycle of *B. cinerea*. When these fungi are exposed to unfavorable conditions, microconidia are an alternative microscopic mechanism of reproduction. They are usually seen in cultures of fungi that are aging or have been previously contaminated with other organisms and are often found with sclerosis. Microconidia are formed from germ tubes produced by macroconidia in the empty cells of mature filaments.

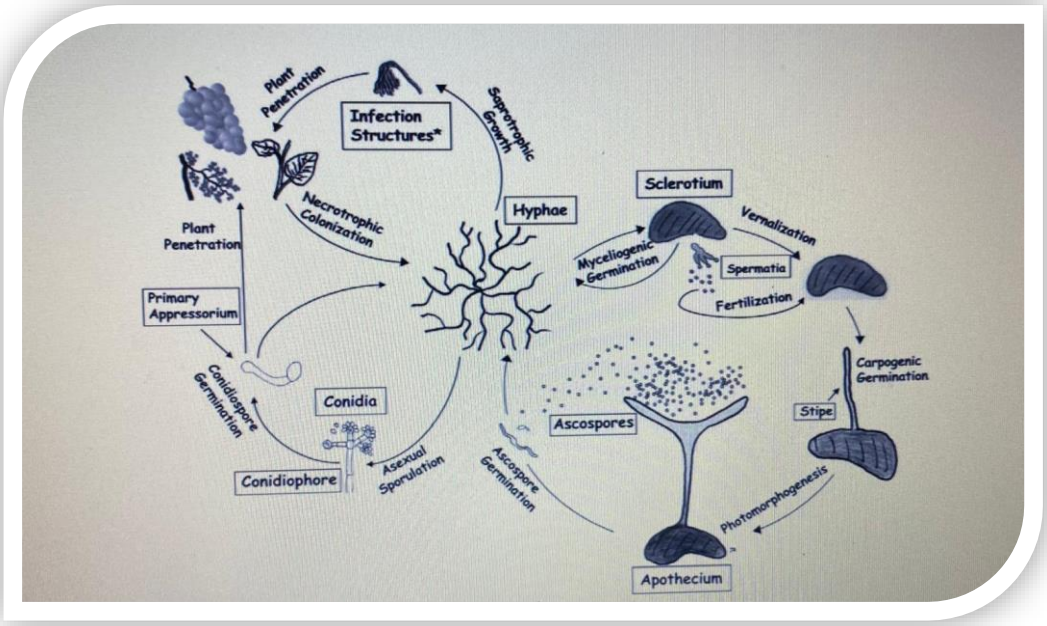


Figure 1.6 : Life cycle *of Botrytis cinerea*, with different stages of sexual and asexual development.

1.6.1.4 Biology of *Botrytis cinerea* infection process

The fungi occur as mycelia, micro-and macroconidia, chlamydospores, sclerotia, apothecia, and aeciospores in various habitats and spread in different ways, the macroconidia of *B. cinerea* being usually released in dry air currents. The life stage of *botrytis* is a somatic, vegetative, or anamorphic stage in which the fungus produces asexual conidia (macroconidia) and hardens under air-dry currents. In senescent cultures, the pathogen produces abundant microconidia of phialids that function primary as spermatia. Sclerotia germinate naturally to form mycelia or conidia, but may also germinate to produce aphotecia (teleomorphic stage) containing ascospores formed by meiosis.

In temperate climates, sclerotia germinate in spring and serve as the main source of inoculum in a crop In addition to sclerotia in winter or during periods unfavorable to the fungus, the mycelium of *B. cinerea* also survives in infected, dead host tissue left as crop residue and in some seeds and serves as the primary inoculum. In perennial plants, the dead leaves, flowers, and mummified fruits contain masses of mycelium that are often ideally placed in the canopy of a plant to produce conidia and initiate infections. *B. cinerea* is a typical example of a necrotrophic fungus: it first kills the cells of the host plant and then colonizes the dead tissue. 0°C is the minimum temperature for growth, 20°C is the optimum temperature, and 30°C is the maximum temperature. As a result, *B. cinerea* thrives in the cold storage of fruits and vegetables. (Romanazzi and Feliziani, 2014).

1.6.1.5 Postharvest factors favoring *Botrytis cinerea* infection

Overripe fruit is more susceptible to gray mold infection, so proper selection of fruit ripeness at harvest is as important as the other cultural practices. During storage, temperature, relative humidity, air velocity, atmospheric composition (oxygen, carbon dioxide, and ethylene concentrations), and sanitation practices all affect the rate of postharvest deterioration. Careful handling and avoidance of mechanical damage or pathophysiological injury caused by excessive cooling or lighting, or by anomalies in

atmospheric gas composition, are fundamental to the control of decay. In many cases, these causes are interrelated, i.e. mechanical injury can be associated with postharvest rot for many reasons. In the food industry, it is common to use technologies during storage (e.g. exposure to modified or controlled atmosphere) to delay fruit respiration and then ripening and senescence to extend fruit shelf life (Romanazzi and Feliziani, 2014).

1.6.1.6 Agronomic and environmental factors influencing *Botrytis cinerea*

Some management can be done, if agronomic choices and environmental conditions are the main aspects that can be artificial modified. In the field, treatments with resistance inducers could activate the plant defenses and prevent the onset of infections. This strategy could be a new methodology to act on the disease triangle on the side of the host. Having a fortified plant could be helpful in reducing the possibility of disease development. Selecting plant varieties that are suitable for the growing area and growing locations that are not suitable for gray mold growth is crucial. When selecting a growing area, look for areas with warm, dry spring weather or areas where it is windy throughout the day. In terms of cultural practices, the most effective way to prevent B. cinerea activity is to minimize high humidity and stagnant air, especially in the fruiting zone, since these are excellent conditions for fungal growth. Leaf removal and proper canopy maintenance can help reduce high humidity. Micro-irrigation should be preferred to canopy irrigation. Planting strawberries outdoors should be planned to ensure optimal air circulation. Plastic mulch can help avoid berry- soil, but be careful not to allow puddles of water to form under the fruit on the plastic. Heating and ventilation can help avoid high humidity in greenhouse farming. This may require further venting early in the early morning hours when the moisture has condensed and the air has been warmed by the sun. (Romanazzi and Feliziani, 2014).

1.6.1.7 *Botrytis cinerea* management

1.6.1.7.1 Fungicides:

Infection with *B. cinerea*, which causes postharvest rot, usually occurs before harvest at the field stage and may remain latent until storage. Traditional control of gray mold infections consists of applying synthetic fungicides to the field during the crop growing cycle. Since there are no effective methods to predict the risk of this disease, preventive applications before the appearance of disease symptoms appear. For example, in table grapes, four fungicide applications are usually made at the end of flowering, at bunch closure, at maturity, and 3 weeks before harvest. In strawberry, fungicides are applied around flowering and repeated until harvest, depending on weather conditions and the pre-harvest interval of the formulations For Botrytis cinerea (gray mold) 139 kiwifruit, some postharvest treatments with fungicides are applied as well as field applications before commercialization, i.e. the fungicide fluodioxonil is registered in Italy as a postharvest treatment to be applied 30 days before consumption (Romanazzi and Feliziani, 2014).

Gray mold infections can be controlled with a variety of synthetic fungicides. Fungicides that influence fungal respiration, microtubule activity, osmoregulation, methionine biosynthesis, or sterol biosynthesis can be classified according to their biochemical mode of action.

1.6.1.7.2 Alternatives to Synthetic Fungicides:

Although the use of synthetic fungicides in the field remains the most common method of controlling postharvest rot of fruits and vegetables, their use has several negative aspects that must be considered. Fungicide residues on fruit decrease after harvest and storage rots may occur. For some commodities, the use of post-harvest fungicides is not allowed due to various normative restrictions and, in general, the use of fungicides is prohibited in organic farming. In addition, increasing public concern about fungicide residues has contributed to increased interest in the development of alternative methods to control postharvest decay of fruits and vegetables, which must be integrated with, if not completely replaced, the use of

synthetic fungicides. The advent of pathogen isolates resistant to one or more fungicides has also reduced the frequency with which they are used. Novel control techniques have been developed as alternatives to synthetic fungicide treatments as a result of research activities. Biological control agents (BCAs), natural substances, compounds generally regarded as safe (GRAS), and physical approaches alone or in combination with all four groups can be grouped into four basic categories for schematic reasons.

1.6.1.7.2.1 Biological control agents (BCAs)

Bacteria and yeasts are representing BCAs that serve as 'antagonists' to pathogens that cause fruit deterioration after harvest. Competition for nutrition and space, antibiosis, parasitism, promotion of resistance in host tissues, and creation of volatile metabolites are all methods they can use. Several products based on microorganisms have been approved for use against B. cinerea.

1.6.1.7.2.2 Physical control:

Heat treatment, UV-C light, hypobaric and hyperbaric treatments, and exposure to a modified or regulated environment or ozone are examples of physical agents. Physical therapies on the fruit can have a dual effect, acting against the pathogen while also generating defensive reactions from the host.

1.6.2 *Fusarium oxysporum f. sp. Lycopersici*:

1.6.2.1 The host rang and importance:

Fusarium oxysporum f. sp. lycopersici (FOL) is a soil-borne pathogen that causes tomato wilting and, in certain cases, considerable yield losses. *Fusarium oxysporum* is a fungus that includes a number of important plant and human diseases as well as toxigenic microorganisms. Fusarium wilt is a disease caused by *Fusarium oxysporum f. sp. lycopersici* (FW). They cause significant losses to essential vegetable crops in the field and in the greenhouse, and they continue to be significant limiting factors for tomato productivity. In susceptible host variegates, FW losses can be very high. India has recently recorded yield losses of up to 45 percent. (McGovern, 2015)

1.6.2.2 *Fusarium oxysporum* symptoms:

Yellowing, stunting, and wilting are symptoms of *F. oxysporum* infection in tomato seedlings. Yellowing and wilting of leaves are common wilt symptoms induced by *F. oxysporum* on mature tomato plants, and they are usually most severe after blooming and fruit set, as well as during the hottest part of the day (see Figure 1.9). Because discrete parts of vascular tissue are affected and inhibited, the symptoms of Fusarium wilt can be unilateral, and vascular discoloration can extend the full length of the stem into the vascular tissue of the petioles. Warm temperatures (28 C), lower soil pH, and the use of ammonium-based fertilizers enhance the symptoms of FW.

Figure 1.7: *F. oxysporum symptoms* on tomatoes: 1) Yellowing and death of leaves on one side of the stem, 2) Dark brown vascular discoloration).

1.6.2.3 *F. oxysporum* lifecycle:

Fusarium species have three stages in their life cycle: dormant, parasitic, and saprophytic. The suppression and germination of soil-borne structures occurs during the dormant stage. Root invasion, colonization of the root cortex and endodermis, migration to the xylem, colonization of the xylem of stems and leaves, manifestation of symptoms, and finally death of the host are all part of the parasitic stage. The saprophytic stage involves the development of the dead host's latent structures. *F. oxysporum* differs in the shape of the macroconid, the structure of the microconidiophores and the formation of chlamydospores. (Beckman 1987).

Under unfavorable conditions the fungus causes sclerosis (singular = sclerotium). A sclerotium is an organized mass of hyphae that becomes dormant under unfavorable conditions and germinates when favorable conditions return and become a source of infection. Macroconidia and microconidia are usually formed from slender phialides by Fusarium species. Macroconidia are hyaline, fusiform to sickle-shaped, two- to multicellular, usually with elongated apical cells and basal pedunculate cells. Microconidia are 1- to 2-celled fusiform, hyaline, pear-shaped, ovoid, straight or curved.

1.6.2.4 *F. oxysporum* management:

To be effective, a plant disease control program must look at the total number of plant-pathogen interactions and either eliminate or change the balance in the plant's favor. Pathogen inoculum viability (population density) and/or functionality (ability to infect the host successfully) must be reduced.

1.6.2.4.1 Chemical control:

1.6.2.4.1.1 Disinfectants

Fol propagules can survive on and in a variety of horticultural surfaces, including irrigation water, containers, supports, and buildings, therefore eliminating them through disinfestation is an important part of their management. Disinfectants (sensu Agrios, 2005) commonly used in agriculture, such as sodium hypochlorite (NaOCl), hydrogen peroxide (H2O2), and ozone (O3), are powerful oxidizers that inhibit the growth pathogens by disrupting protein and nucleic acid structure and/or function; other disinfectants, such as alcohols (ethanol, isopropyl alcohol), and quaternary ammonium salts, cause protein denaturation

1.6.2.4.1.2 Fungicides

Despite the fact that a large range of fungicides is active against *Fol*, they are employed less frequently than fumigants and other disease control strategies, with the exception of greenhouses in some countries. Bromuconazole and prochloraz, when applied as soil drenches at 10 mg ai/ml, were more effective in reducing the severity of FW than azoxystrobin, benomyl, carbendazim, and fludioxonil at the same rate, however, all fungicides tested significantly reduced FW compared to the control.

1.6.2.4.1.3 Fumigants

Pre-plant fumigation has become the most frequent management approach for FW, specifically in North America and Europe, after resistance. The standard treatment for these and other soilborne diseases, nematodes, and weeds has been bromomethane (methyl bromide) in various combinations with trichloronitromethane (chloropicrin); however, methyl bromide is being phased out in accordance with the Montreal Protocol because it is an ozone-depleting substance. As a result, throughout the past two decades, the key motivation in agricultural fumigation research has been the detection of methyl bromide alternatives. Several fumigants, including 1,3- dichloropropene chloropicrin, chloropicrin, methyl isothiocyanate, propylene oxide, and sodium azide, have been evaluated in comparison to methyl bromide chloropicrin against Fol in multiple experiments, mostly in Florida, USA. Except for propylene oxide, all fumigants consistently reduced FW to levels comparable to methyl bromide chloropicrin; 1,3-dichloropropene chloropicrin also consistently produced yields comparable to methyl bromide chloropicrin.

1.6.2.4.1.4 Biological soil disinfestation

Anaerobic soil disinfestation (ASD), also known as biological soil disinfestation, involves applying organic matter and other labile carbon to the soil surface, followed by irrigation and polyethylene mulching, to generate microbial-driven anaerobic soil conditions that inhibit soil-borne pests such as fungi, bacteria, nematodes, and weeds.

1.6.2.4.1.5 Plant nutrition and soil chemistry

Plant nutrition can affect their susceptibility to disease. Furthermore, soil pH and nitrogen type in fertilizers have long been known to alter a plant's susceptibility to diseases *like F. oxysporum* wilts. Applying calcium hydroxide to tomato plants reduced the incidence and rate of FW development, and they attributed this to a rise in soil pH (7.5 or 8.0), rather than increased calcium buildup. (The type of soil amendment had no effect on the calcium content of tomato tissue).The availability of micronutrients (iron,

manganese, and zinc) required by *F. oxysporum* diminishes as soil pH rises. They were able to manipulate soil pH and nutrients to create favorable and unfavorable circumstances for wilt; low pH, high NH4eN, high P, high Mg, and all given micronutrients promoted wilt, whereas high pH, high NO3eN, low P, low Mg, and omission of iron, manganese, and zinc reduced wilt.(Jones and Woltz 1968)

1.6.2.4.2 Biological control

Antibiosis, competition for nutrients (especially Fe through bacterial siderophore production) and colonization sites, induced resistance, and hyperparasitism /predation are just a few of the mechanisms that can occur between biocontrol agents and Fol, as well as interactions between soil microflora in general.

Biological control of FW has included a diverse spectrum of microbes, both alone and in combination. Different isolates of the same bacterial species, different bacterial genera, different fungal genera, and combinations of bacteria and fungi were among the biocontrols tested against Fol. Antibiosis was used by a number of biocontrols to diminish spore formation, germination, and/or survival; competition and induced resistance were also identified in recent investigations. Biocontrols reduced FW symptoms and enhanced yields in general, and in some cases outperformed conventional fungicides. Combinations of biocontrol agents were more successful than their separate components in the majority of situations. This finding implies that increasing microbial diversity in plant disease treatment is advantageous.

1.6 Bioactivity

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

Pharmaceuticals, food additives even on natural pesticides sectors have become interested in bioactive molecules from natural sources (Anklam et al., 1998; Ambrosino et al., 1999). Compounds can be biologically identified and distinguished from different plant parts such as leaves, flowers, and fruits. It remains with other compounds found in plants

The use of plants for human consumption has a long history dating back to the dawn of time. Originally, plants were used for nutritional purposes, but after their therapeutic properties were discovered, this natural flora became a valuable source for curing diseases and improving the health of people around the world. The history of bioactive compounds can be traced back to the use of herbal plants in ancient times. At that time, people did not know what bioactive chemicals were, although there were numerous applications in various fields.

The history of bioactive compounds can be traced back to the utilization of herbal plants in ancient times. People had no understanding what bioactive chemicals were in the past, although their applications were numerous in various fields.

Typically, bioactive compounds of plants are produced as secondary metabolites. Every living body, from one cell bacterium to million cell plants, processes diverse chemical compounds for their survival and subsistence. All compounds of biological system can be divided into two broad arenas. One is primary metabolites, which are the chemical substances aimed at growth and development, such as carbohydrates, amino acids, proteins and lipids. Another is secondary metabolites, which are a group of compounds other than primary metabolites believed to help plant to increase their overall ability to survive and overcome local challenges by allowing them to interact with their surroundings (Harborne, 1993).

Plant bioactive compounds are substances produced by plants that have pharmacological or toxicological effects in humans and animals. Although nutrients (e.g. vitamins and minerals) have pharmacological or toxicological effects when consumed in large doses, nutrients in plants are often not included in the 12 term bioactive plant component. Secondary metabolites are the main sources of bioactive chemicals in plants. (Askala 2010)

Aside from the principal biosynthetic and metabolic routes of substances directed at plant growth and development, such as carbohydrates, amino acids, proteins, and lipids, secondary metabolites are created within plants. They can be thought of as products of biochemical "side tracks" in plant cells that aren't required for the plant's regular functioning. (Askala 2010).

1.7 Bio-pesticide:

Biopesticides are pesticides that are made from natural elements such as animals, plants, microbes, and minerals. Canola oil and baking soda, for example, have pesticidal properties and are considered biopesticides. There are 299 active biopesticide components and 1401 active bio pesticide product registrations as of April 2016 (US EPA, 2015).

1.8.1 Classes of Biopesticides

Biopesticides fall into three major classes:

1. Biochemical pesticides are naturally occurring compounds that use non-toxic ways to control pests. Conventional pesticides, on the other hand, are mainly synthetic chemicals that kill or inactivate the bug directly. Pesticides that interrupt mating, such as insect sex pheromones, and various scented plant extracts that draw pests into traps are examples of biochemical pesticides. Because determining whether a compound fits the criteria to be designated as a

biochemical pesticide can be complex, the EPA has established a special committee to make such determinations.

2. Microbial pesticides: the active ingredient in microbial pesticides is a microbe (e.g., a bacterium, fungus, virus, or protozoan). Although each active ingredient is somewhat unique to the target pest, microbial pesticides can control a wide range of pests (s). Fungus that control weeds, for example, and fungi that kill insects are two examples.

3. Plant-Incorporated-Protectants (PIPs) are pesticidal compounds produced by plants from genetic material that has been introduced to the plant. Scientists can, for example, integrate the gene for the Bt insecticide protein into the genetic material of plants. The chemical that kills the pest is then manufactured instead of the Bt bacteria. The EPA regulates the protein and its genetic material, but not the plant itself.

1.8.2 Advantages of using bio-pesticides:

The use of biopesticides has a number of advantages, including the fact that they are typically less harmful than conventional pesticides. In contrast to broad-spectrum, conventional insecticides, which can impact organisms as diverse as birds, insects, and mammals, they generally affect only the target pest and closely related organisms.

furthermore, biopesticides are frequently effective in little amounts and degrade quickly, resulting in reduced exposures and essentially avoiding the pollution problems that conventional pesticides produce. Biopesticides, when utilized as part of Integrated Pest Management (IPM) programs, can significantly reduce the usage of conventional pesticides while maintaining good crop yields.

1.9 Objectives:

There is a trend towards natural fungicides (bio-fungicides) to ensure clean soil, water, environment, and plants, also to preserve human health. These bio-fungicides extracted from available, cheap, safe `1and renewable natural sources such as *T. persicus*, *Achillea fragrantissima*, *salvia dominica* and *Varthemia iphionoide* which are what our research aims for.

1. To characterize the polyphenolic compounds in the ethanolic extracts of *Thymus persicus, Achillea fragrantissima*, *Varthemia iphionoide*, and *Salvia Dominica* leaves using HPLC method.

2. To evaluate the antifungal activity of *Thymus persicus, Achillea fragrantissima*, *Varthemia iphionoide*, and *Salvia dominica* leave extracts against *B. cinerea* and *F. oxysporum*.

Chapter two: Literature Review

2.1 Introduction:

It is considered the discovery of ancient plants since the time of mankind. In the beginning, plants are used in the beginning, as cosmetics, medicinal preparations, and preservatives (Venatoro, Medicinal Products and Preservatives 2001). In the Greek and Roman time period, the timeline goes back to previous search engines for herbal plants including Hippocrates, Theophrastus, Dioscorides, and many more Others (Paulsen, 2010). The Romans have been famous for their use of medicinal herbs for a very long time. For example, Herodotus (5th century BC) mentioned that Leonoroscardia (mother plant) was used by people living north of the Danube in his writings. In the 19th century, the Romanian pharmacopeia introduced herbal products and in 1904 the first Institute of Medicinal Herbs was established in the city of Cluj (Vinatoro, 2001). The use of herbal plants in antiquity actually illustrates the history of biologically active molecules. In the past, people had no idea what biologically active molecules were but the use of these compounds was diverse enough in different possibilities.

2.2 Natural products:

(Villaverde et al., 2016) show biomass is important as a source of biofuels, energy, and widely accepted chemicals. At present, the main concern is the valorization of the by-products of lignocellulosic materials. Chemical compounds are extracted from plants and microorganisms to provide protection for crops from pests, weeds, and diseases (active substances in biocides) for the production of pesticides. Their frequent use is encouraged by new pesticide regulations that discourage the use of harmful active substances. . This article presents the current and future status of bio-pesticides, and draws on the European pesticide legislative framework for its potential. The article also talks about the importance of different methods/mechanisms for the production of good materials that are produced from natural sources, the role of chemistry in the development of bio-pesticides , and how to build management practices in an increase in bio-pesticides.

The study of (Marrone, 2019) indicates that there is a long history of producing new pesticides from natural products, but there is still a relatively small percentage of pesticides taken naturally compared to the pesticides obtained from natural sources. Relying on the US Environmental Protection Agency (EPA), bio-pesticides have been around for 70 years, starting with Bacillus thuringiensis, and the good thing is that they are becoming the best and most science-based products that are growing rapidly. There are many restrictions on chemical synthetic pesticides. Biocides make up about $3-4 billion of the $61.3 billion pesticide market, which is a small percentage. But the growth of bio-pesticides is expected to exceed that of chemical pesticides, with annual growth rates between 10% and 20%. Biocides when integrated into crop production and pest management programs provide the ability to increase crop yields and quality when compared to chemical programs only. Among the added benefits: eliminating or reducing chemical residues, facilitating the export process, enabling delays in the development of resistance to pests and pathogens, biodegradability and production through agricultural raw materials versus fossil fuels, reducing risks to non-target organisms and ensuring pollinators. Challenges to the use of bio-pesticides Lack of awareness and education of how to deploy their rare modes of action in an integrated program, testing of products alone versus integrated programs, and long-term perceptions of cost and efficacy.

2.3 Medicinal plant :

In Jordan, Qaisum (Fragrantissima pies) is utilized by (Al-Rawashdah, 2009) study . It is the only variety of aquelia that is used to treat diabetes, lower blood cholesterol, and as a carminative, intestinal colic, dysmenorrhea, and different illnesses.

Varthemia iphionoides is native to Palestine, according to a study (Salameh et al., 2011), and it is traditionally used to treat digestive issues. The goal of this study is to confirm the pharmacodynamic effect of *Varthemia iphionoides* in medicinal applications. On isolated ileum of rabbits, ethanol and water extracts of *Varthemia iphionoides* were tested for antispasmodic activity. The aqueous extract of *Varthemia iphionoides* considerably reduced the increase in ileus generated by the acetylcholine (ACh) concentration of 5.5X10-6M in this investigation, demonstrating that its

principle of action is concentration-dependent. This suggests that the *Varthemia iphionoides* extract works by inhibiting muscarinic receptors at the very least.

2.4 *Botrytis cinerea*:

Ben-Jabeur et al., 2015 explain that the ability of thyme essential oil to induce systemic acquired resistance in hydroponically grown tomatoes and their seedlings were found in rice and fusarium raspul. Thyme oil was discovered by 64% of the colonization of Botrytis cinerea on central leaves. In 7 days after treatment, it is reduced to 30.76%. The accumulation of phenolic compounds and peroxidase activity was examined, to reveal the pathways of plants produced in response to thyme oil, and it was observed in response to the plant after feeding the roots in hydroponics or after foliar spraying. When applied to the roots.

Botrytis cinerea is one of the most important diseases of conventionally farmed strawberries, according to a study (Daugaard, 1999), and it is treated by chemical fungicides during the flowering period. We need to explore alternatives to fungicides in organic farming. This review examines culture approaches for controlling *B. cinerea* bacteria in strawberries, concluding that no one or combination of methods is capable of preventing the disease. A dynamic, adaptable approach is needed to manage a non-fungicide-free agro-ecosystem for *B. cinerea*. More research is needed before any judgments about its effectiveness in controlling *B. cinerea* can be reached.

Fukumori et al. (2004) state microconidia can reproduce by sexual reproduction by the sperm of the bacterium cinerea. Through experiments, when muscle stiffness from one isolate mate with microconidia from another isolate, sexual bodies are formed. Initially the otica that form on the sclera are deeply concave or funnel-shaped; and expand the heads, and eventually turn into a flat and convex. Sizes range from 10 to 15 mm in height and 5 to 7 mm in head diameter. ASCII is shaped into a preservative from the base of the pharmacy caps. They are cylindrical, vitreous and capricious at maturity and contain eight ascospores.

2.5 Gray mold

(Badawy and Rabea, 2009) study showed that the most important post-harvest disease of fruits and vegetables during harvest and during storage in economic terms is *Botrytis cinerea (Pers.)* This study was conducted to verify the effectiveness of chitosan with various molecular weights on gray mold in vivo in tomato fruits stored at different temperatures (Solanum). *lycopersicum L. var. lycopersicum)* and in vitro, the results showed that the antifungal activity increased with decreasing the molecular weight of chitosan. In vivo, chitosan treatments significantly reduced the decay of fungi, and all compounds at concentrations of 2000 and 4000 mg/L showed complete control of fungi in the inoculated fruit with the wound. Chitosan with a molecular weight of 5.7 x 104 g/mol is the most potent compound among those tested. And that a high concentration of chitosan is associated with a lower incidence of disease regardless of storage conditions.

(Yusoff et al., 2020) study, reported that *Botrytis cinerea*, which causes gray mold, which is a harmful disease in tomatoes after harvest, is known to be a limiting factor for tomato production. In the crude extract which had the highest antifungal activity. The results showed that among all the crude extracts, the crude extracts of hexane, dichloromethane, methanol and water extracts at concentration levels at 100, 200, 300, 400 and 500 mg/ml had the most effect on inhibiting *B. cinerea* bacteria. It showed that Dichloromethane extract is the most effective in terms of antifungal activities. For the in vivo bioassay, fruits treated with dichloromethane extract at 400 and 500 mg/ml showed the lowest disease incidence with mild severity of infection. Twenty-three chemical compounds were identified in the p-amygdalina dichloromethane extract using GCMS analysis. The top five major compounds were dominated by squalene (16.92%), phytol (15.05%), triacontan (11.31%), heptacosan (7.14%), and neovitadine (6.28%). This study demonstrated that gray mold disease on tomato fruits is inhibited by V. amygdalina from dichloromethane extract and has abilities as a natural antifungal agent.

2.6 *Fusarium oxysporum*

A 2015 study in McGovern, tomato (Solanum Lycopersicum) induced by *Fusarium oxysporum f. s. lycopersici* and *F. oxysporum* f. s. radicis-lycopersici, respectively, Most recent research focuses on the management of FW and FCRR with diverse individual strategies and their integration including host resistance, and biological, physical, and chemical control.

Bin Jaber and others conducted a study at 2015 showed an evaluation of the capabilities of thyme essential oil to control gray mold and fusarium wilt and to induce systemic acquired resistance in tomato and tomato seedlings grown in hydroponics. The study showed that thyme oil significantly reduced the colonization of *Botrytis cinerea* on pre-treated detached leaves compared to untreated controls. The severity of fusarium wilt significantly decreased, especially in 7 days after treatment when it was reduced to 30.76%. The accumulation of phenolic compounds and peroxidase activity were examined to explore the plant pathways produced in response to thyme oil, the plant 2.response either after foliar spraying or feeding roots in hydroponics which is mostly attributed to the accumulation of peroxidase rather than the accumulation of phenolic compounds, thyme oil is more effective when applied to the roots.

Chapter three: Methodology

3.1 Materials and equipment's

All materials used in this project are from the laboratory of Al-Quds University and Biotech Medical Supplies Company.

i. Materials and equipment used for sample processing

Plant leaves of (*Salvia dominica, Varthemia iphionoide, Achillea fragrantissima,* and *Thymus persicus*), water, ethanol (99%), distilled water, flasks, hot plates, separating funnels, and rotary evaporators are available at Al-Quds University laboratories.

ii. Materials and equipment are used for analysis

Potato dextrose agar, chloramphenicol, plant leaves, ethanol, plastic Petri dishes (50mm), sterilized microtiter plates, autoclaved bottles, germination plates, and incubator.

3.2 Sample Collection:

Our plant species were collected in the summer of 2019 from different locations in Bani Na'im, which is 5 kilometers east of Hebron city.

3.3 Sample preparation:

The plants of khowwekha, shtaila, qaisoom and zaatar were collected and then washed with water to remove any traces of dust. To stabilize the by-product, they were all air-dried in the shade at room temperature to reduce their moisture content and prevent microbial fermentation and subsequent degradation. Then the leaves were separated from the stems and the dried leaves were grounded to obtain a fine powder. The powder was stored in plastic containers at room temperature away from the sun and then used the procedure of extraction explained in (Figure 3.1).

3.4 Simple green extraction method (Extraction of leaves):

50 grams of fine powder of *Salvia dominica* (khowwekha), *Varthemia iphionoide* (shtaila), *Achillea fragrantissima* (Qaisoom) and *Thymus persicus* (zaatar*)* leaves powder was macerated in 500 ml of ethanol (99%) at 40°C and soaked in a beaker covered with perforated aluminum foil for 24 hours. The extracts were then filtered through a Whatman filter paper. The supernatants were combined and concentrated on a rotary evaporator at 40°C under vacuum to give a paste of (khowwekha, shtaila, qaisoom and zaatar*)* leaves extract. The resulting crude extracts of the leaves were stored at -4° C refrigerator in dark bottles at Al-Quds University laboratory as shown in (Figure3.1).

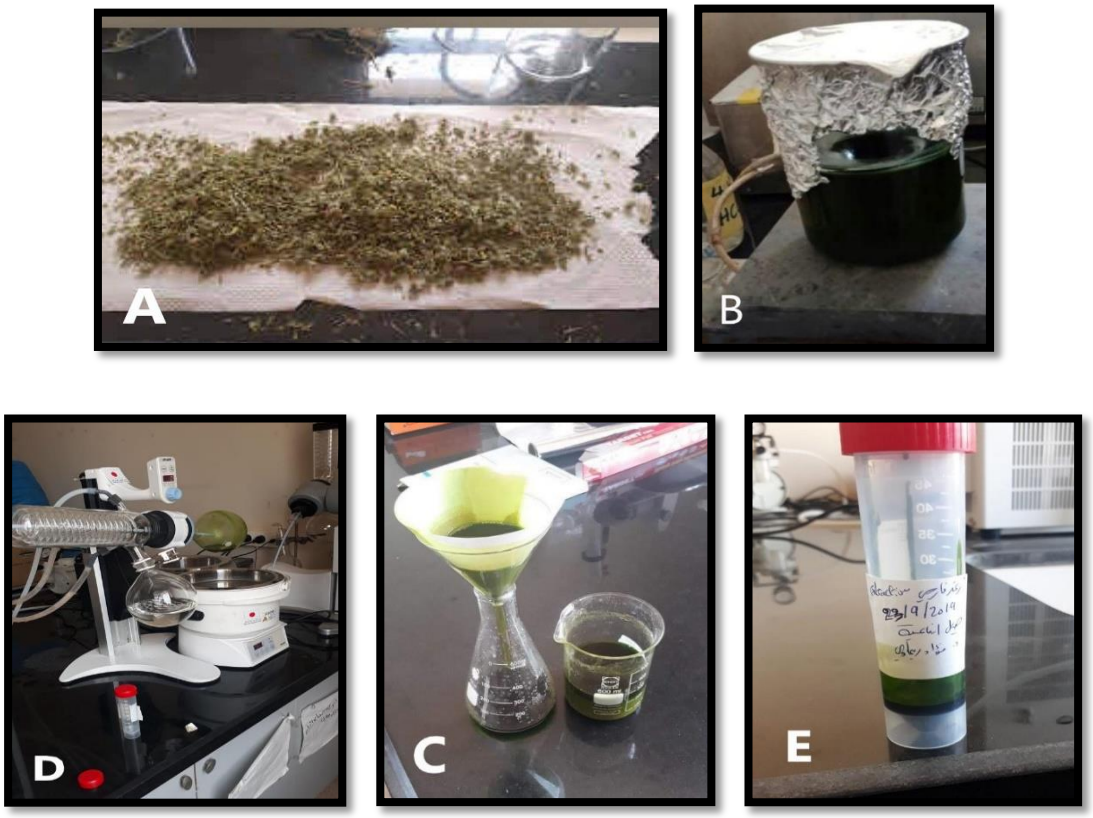


Figure 3.1: Extraction procedure "(A) Dried leaves of plant, (B) leave extracts with ethanol, (C) Extract filtration by Whatman filter paper, (D) Evaporation of leaves extract using a rotary evaporator, (E) crude plant leaves extract".

3.5 RP-HPLC analysis of phytochemicals

HPLC Waters Alliance e2695 equipped with 2998 PDA detector was used for the analysis of bioactive compounds. Data acquisition and control were carried out using Empower 3 chromatography data software.

3.5.1 Quantification of phenolic compounds

Reversed phase HPLC method was employed for analysis of different polyphenolic compounds and flavonoids using C18 column (25 cm with 3.6 µm inner diameter) and a mixture of 0.5% acetic acid (solution B) and Acetonitrile (solution C) with a linear gradient mode according to the table below (Table 3.1), with a flow rate of 0.5 mL/ min and Column temperature of 25 ºC and injection volume of 20 µL. All samples were filtered with 0.45 µm disposable filter. Photodiode array detectore with wavelength range of 210 - 400 nm was employed.

Table 3.1. The gradient mobile phase conditions used for RP-HPLC analysis of the polyphenolic compounds and flavonoids in the plant extracts, 0.5% acetic acid (solution B and Acetonitrile (solution C).

Time (minutes)	B %	C %
0.0	95%	5%
50.0	80%	20%
65.0	65%	35%
70.0	40%	60%
75.0	10%	90%
78.0	95%	5%
80.0	95%	5%

3.5.2. Preparation of standard solutions

The followings are the standards that used in HPLC analysis: gallic acid, caffeic acid, syringic acid, trans cinnamic acid, 3,4-dihydroxybenzoic acid, 3,4-dihydroxyphenylacetic acid, 4-hydroxyphenylacetic acid, rutin, ferrulic acid, quercetin,

vanillic acid, isovanallic acid, kampferol, chlorogenic acid, verbacoside, Sinapic acid, and p-coumaric acid. 5mg of each standard was dissolved in 5 ml of 20% ethanol and volume was made up to 25 ml by distilled water. The mixture of the standards were injected (20 µL) into the HPLC chromatograph and analyzed using the RP-phase method described above.

3.5.3. Preparation of Samples of the plant extracts for HPLC test:

About 100mg accurately-weighed dry extract was transferred to a 100mL volumetric flask. 20mL 95% ethanol was added and sonicated and volume was made up to 100mL by distilled water.

3.6 In Vitro Assays (Mycelial Growth Rate)

Mycelia Growth Rate is used to evaluate the antifungal activity of plant leaves extracts, at different concentrations. The effect of *Salvia dominica, Thymus persicus, Varthemia iphionoide,* and *Achillea fragrantissima* extracts on the mycelial growth rate of the two fungi was studied in vitro using 4 g of potato dextrose agar (PDA) and 0.1 g of chloramphenicol. Each flask containing 100 ml distilled water and 4 g PDA medium is placed on a hot plate with magnetic stirrer to dissolve and homogenize the components; the flasks are then autoclaved and allowed to cool to 55˚C - 60˚C. The extracts were prepared and added to the growth media to obtain the final concentration of plant extract (0.5%, 1% and 2%). 14 ml of growth media was added to each Petri plate (90 mm diameter) (Figure 3.2). The experimental design was completely randomized (CRD) with 3 Petri plates (replicates) for each plant extract concentration for each *B. cinerea* isolate and *F. oxysporum*. The amended Petri plates were then inoculated with 5 mm mycelial disks from 5-day-old cultures of the *B. cinerea* and *F. oxysporum* isolates.

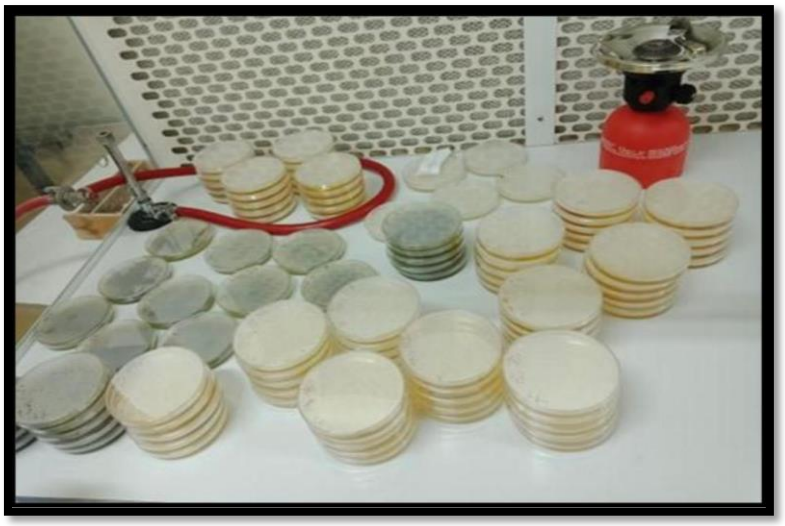


Figure 3.2: Preparing of PDA media.

Plates were then incubated in the growth chamber at 25°C. Colony diameters were measured after 3 and 6 days and the growth rate of mycelium (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).

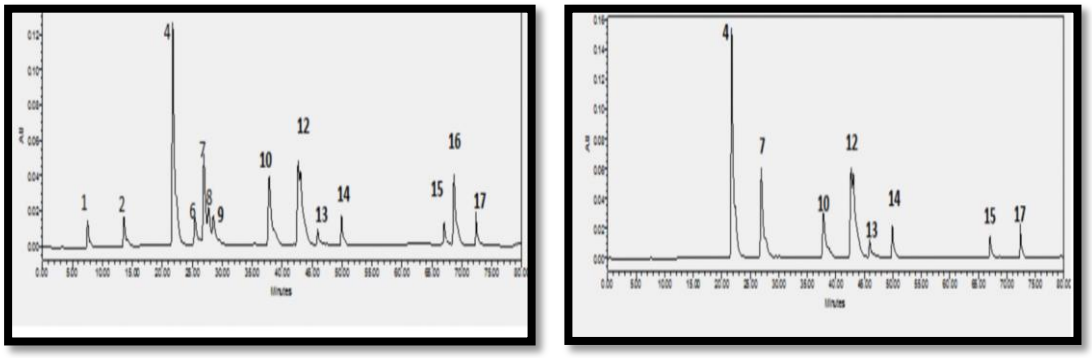
Where: R: mycelium growth rate. D: average diameter of the colony (cm) after 6 days, d: average diameter of the colony (cm) after 3 days, T: time of incubation (day)

Chapter four: Result and Discussion

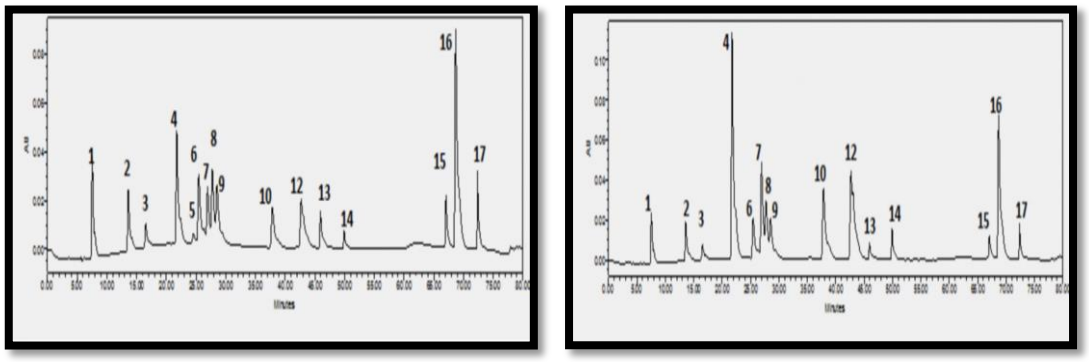
4. Results

4.1. HPLC analysis of the standards of polyphenolic compounds and flavonoids

The mixture of 17 standards were injected (20 µL) into the HPLC chromatograph and analyzed using the RP-phase method described above. Different wavelengths using the photodiode array detector was used as each compound has its own wavelength of maximum absorption (Table 4.1). Figure 4.1 shows the chromatograms of the standards mixture at different wavelengths (300 nm (a), 323 nm(b), 270 nm (c), and 290 nm (d)). As it is obvious from Figure 3.1 (a-d), the 17 compounds were separated when different wavelengths were used. Table 4.1 summarizes the retention times of the standards with maximum wavelength of absorption for each standard.



a)300 nm b)323 nm



c) 270 nm d)290 nm

Figure 4.1 HPLC chromatogram of polyphenolic and flavonoid standards analysed using RP-HPLC method at 300 nm (a), 323 nm(b), 270 nm (c), and 290 nm (d).

Table 4.1. List of Standard compounds analyzed using RP-HPLC method with their retention times and maximum wavelength of absorption.

Standard #	Standard name	Retention time (min)	Wavelength (nm)
1	Gallic acid	8.26	271
2	3,4-Dihydroxybenzoic acid	13.87	259
3	3,4-Dihydroxyphenylacetic acid	16.57	280
4	Chlorogenic acid	21.64	323
5	4-hydroxyphenylacetic acid	24.55	274
6	Vanallic acid	25.42	260
7	Caffeic acid	26.92	322
8	Syringic acid	27.73	274
9	Isovanallic acid	28.55	259
10	p-Coumaric acid	37.82	309
11	Ferrulic acid	42.68	322
12	Sinapic acid	43.1	323
13	Rutin	45.99	255
14	Verbascoside	49.98	329
15	Quercetin	67.04	364
16	Trans-cinnamic acid	68.69	275
17	Kaempferol`	72.36	265

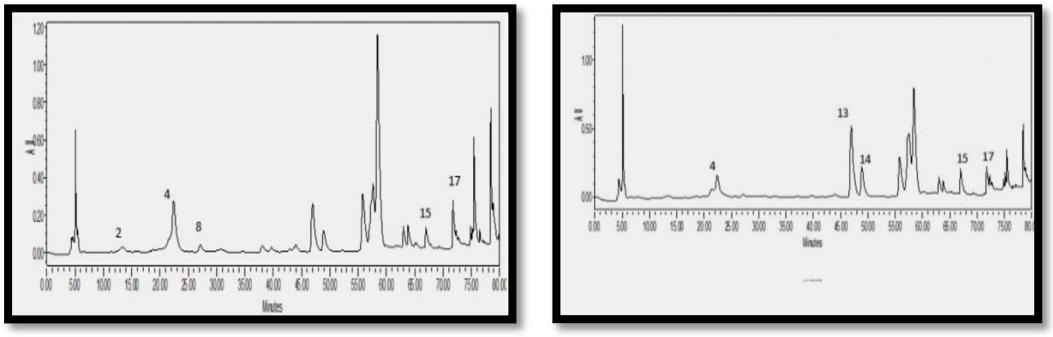
4.2 HPLC analysis of plant extracts

The plant extracts were analyzed using the method developed for the standards. Figure 4.2 shows the chromatogram for *Salvia dominica* ethanolic extract at 2 wavelengths (300 and 250 nm). At 300 nm, 3,4-dihydroxybenzoic acid, kapmpferol, chlorogenic acid, quercetin and syringic acid were detected in the plant extract, while at 250 nm, rutin, verbascoside, kampferol, quercetin and chlorognic acid were detected in the chromatogram of *Salvia dominica* ethanolic extract.

Figure 4.3 shows HPLC chromatogram of *Achillea fragrantissima* analysed using RP-HPLC method at 300 nm. The following compounds were identified in this extract: rutin, chlorogenic acid, kampferol, quercetin, trans-cinnamic acid, sinapic acid, and p-coumaric acid.

Figure 4.4 shows HPLC chromatogram of *Thymus persicus* analysed using RP-HPLC method at 300 nm. Rutin, quercetin and kampferol were identified in this extract.

Figure 4.5 shows HPLC chromatogram of *Varthemia iphionoide* analysed using RP-HPLC method at 300 nm. Rutin, chlorogenic acid and kampferol were identified in this extract.



(a)300 nm b)250 nm

Figure 4.2: HPLC chromatogram of *Salvia dominica* analysed using RP-HPLC method at 300 nm (a), (b)250 nm.

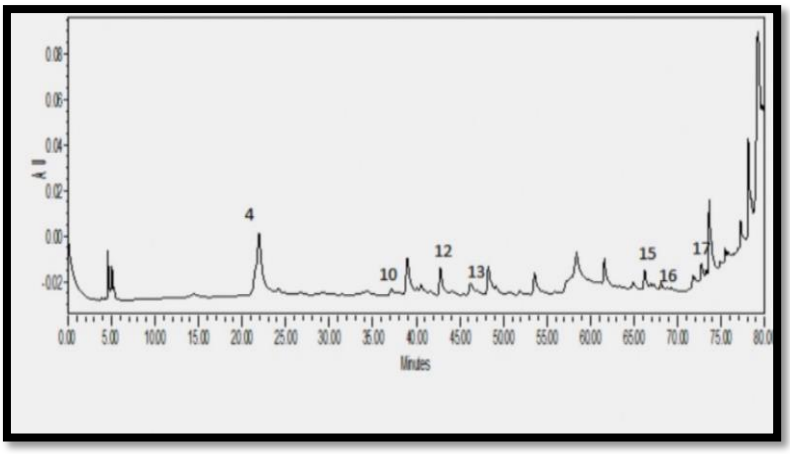


Figure 4.3: HPLC chromatogram of *Achillea fragrantissima* analysed using RP-HPLC method at 300 nm.

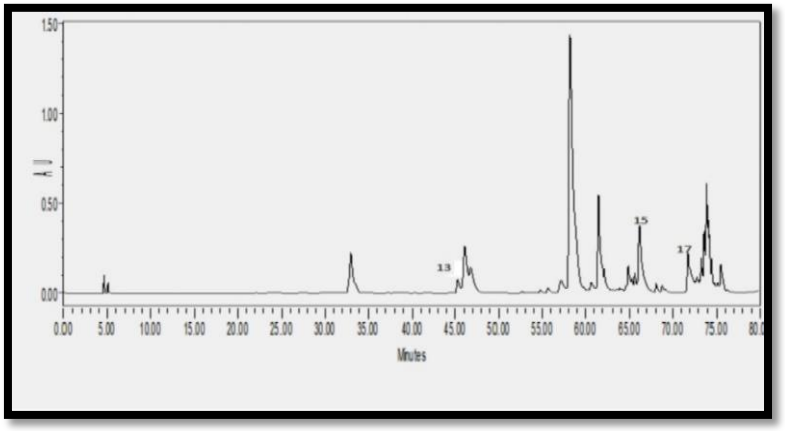


Figure 4.4: HPLC chromatogram of *Thymus persicus* analysed using RP-HPLC method at 300 nm.

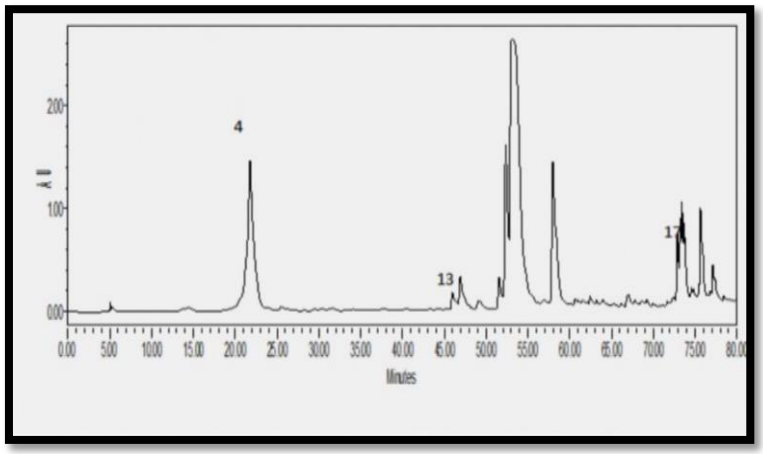


Figure 4.5: HPLC chromatogram of *Varthemia iphionoide* analysed using RP-HPLC method at 300 nm.

4.3 In Vitro Assays:

This section shows the effect of *S. dominica, V. iphionoide, A. fragrantissima* and *P. thyme* against *B. cinerea* and *F. oxysporum* in vitro, including the growth rate of mycelia at different concentrations.

4.3.1 Effect of the studied extracts against *Botrytis cinerea* and *F. oxysporum*:

4.3.1.1 Effect of *S .dominica* extract:

The effect of *S. dominica* leaves extract on the mycelia growth rate of mycelia of *B. cinerea* and *F. oxysporum* was studied in vitro using potato dextrose agar (PDA) medium. Three media samples with different concentrations were prepared for each *S.dominica* leaves extract. The volume of each sample was 100 ml. The effect *of S. dominica* against *B. cinerea* and *F. oxysporum* is clear in figure 4.6 and figure 4.7.

Figure 4.6: Effect of extract of *S. dominica* on Mycelium Growth of *B. cinerea* at different concentrations (0%,0.5 %,1%,2%).

Figure 4.7: Effect of extract of *S. dominica* on Mycelium Growth of *F. oxysporum* at different concentrations (0%,0.5 %, 1%, 2%).

The results show that the extract of *S. dominica* leaves has antifungal effect on mycelial growth of *B. cinerea* and *F. oxysporum*. The extract of *S. dominica* leaves significantly reduced the mycelial growth of *F. oxysporum* at 0.5%-1% concentration and significantly at 2% concentration. However, the growth of *B. cinerea* gradually decreased when the extract of *Salvia dominica* leaves was added at a concentration of 0.5, 1 and 2%. The reduction in mycelial growth correlated positively with increasing extract concentrations. The results of the effect of extract of *Salvia dominica* leaves against *B. cinerea* and *F. oxysporum* were measured. Subsequently, MGR was calculated and illustrated by statistical analysis, as shown in table (4.2).

Table 4.2: Statistical analysis of the effect of *S.dominica* extract at different concentrations on mycelial growth rate (R) against *B. cinerea* and *F. oxysporum.*

Fungi	**Concentration AS**				**Test statistic**	**p-value**
	0.0%	**0.5%**	**1%**	**2%**		
B. cinerea	9.74 ±0.00 a	3.73 ±0.34 ab	2.91±0.65 ab	2.84±0.32 b	9.596†	0.022**
F. oxysporum	13.76±0.00 a	6.10 ±1.51 ab	5.57±0.48 ab	1.93±1.72 b	9.492†	0.023**

†: Comparison of means using Independent samples Kruskal Wallis test.

- Different letters within row indicate a significant difference at the level 5%, the value represent means ± SD

- a and b, these letters indicate a statistically significant difference at a concentration of 0% and 0.5%

As shown in table (4.2), there is a significant difference in the rate of reduction of *Botrytis cinerea* by the concentration of *Salvia dominica* at a significant level of 0.5%. Also, the MGR of *Botrytis cinerea* had the lowest value at 2% concentration. Kruskal-Wallis test for independent samples shows that there is a significant difference in MGR of *Botrytis cinerea* between the 2% concentration and 0% concentration. In addition, there are small differences in the mycelial growth rate of *Botrytis cinerea* between the concentrations of *Salvia dominica* extracts (0.5%, 1%, 2%). The statistical analysis of the effect of *B. cinerea* on MGR is illustrated by the diagram (4.8).

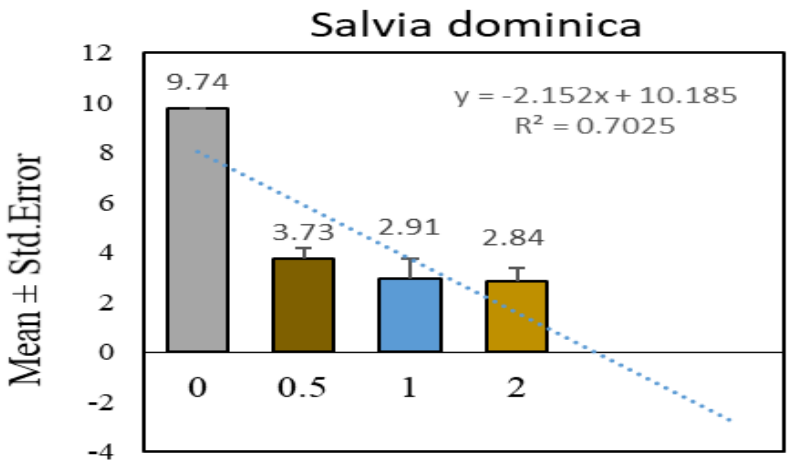


Figure 4.8: Effect of *S. dominica* leaves extract at different concentrations on the Mycelium Growth Rate (R) of *B. cinerea*.

Figure (4.8) shows that there is a negative relationship between MGR of *Botrytis cinerea* and the concentration of *S. dominica* extract concentration, that's mean increasing of extract concentration of *S. dominica* extract reduces the growth of *Botrytis cinerea*. However, a 1% increase in the concentration of *Salvia Dominica* reduces the mycelium growth rate of *Botrytis cinerea* by 70.25%.

On the other hand for the MGR of *F. oxysporum*, results showed that there is a significant difference in MGR of *Fusarium oxysporum* as the concentration of *Salvia dominica* concentration at 5% significant level. The rate of mycelium growth of *Fusarium oxysporum* had the lowest value at 2% concentration. Independent samples kruskal Wallis test indicates there is a significant difference of the rate of mycelium growth of *Fusarium oxysporum* between 2% concentration and 0% concentration. In addition, there are small differences in the rate of growth of *Fusarium oxysporum* between *Salvia dominica* concentrations (0.5%, 1%). The statistical analysis of MGR of *F. oxysporum* is illustrated in Figure (4.9).

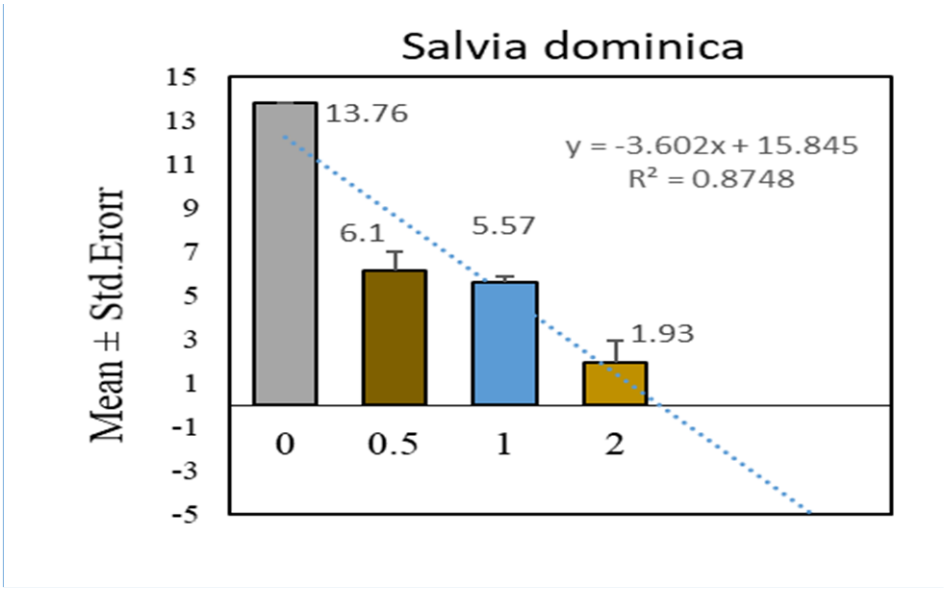


Figure 4.9: Effect of *S. dominica* leaves extract at different concentrations on the Mycelium Growth Rate (R) of *F. oxysporum.*

Figure (4.9) indicates that there is negative relationship between MGR of *Fusarium oxysporum* and *S. dominica* extract concentration, that's mean increase in extract concentration of these medicinal plant reduce the growth of *Fusarium oxysporum*. Furthermore, increasing *Salvia dominica* concentration by 1%, reduce the growth of *Fusarium oxysporum* by 87.48%.

4.3.1.2 Effect of *Varthemia iphionoide* leaves extract:

The effect of *V. iphionoide* leaves extract on the mycelial growth rate of *B. cinerea* and *F.oxysporum* was evaluated in vitro by using a potatoes dextrose agar (PDA) medium. *V. iphionoide* leaves extract, for each one of the three media samples were prepared with different concentrations, the volume of each sample was 100 ml, which the effect of *V. iphionoide* was shown in figure 4.10 against *B. cinerea* and figure 4.11 against *F. oxysporum*.

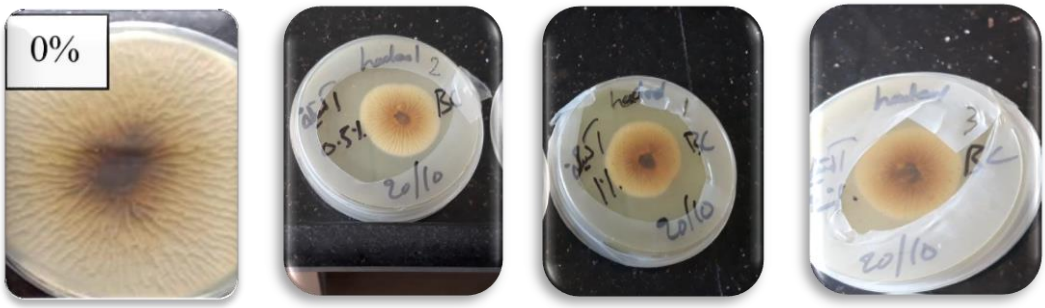


Figure (4.10): Effect of *V.iphionoide* extract on Mycelium Growth *of B. cinerea* at different concentrations (0%,0.5%,1% and 2%).

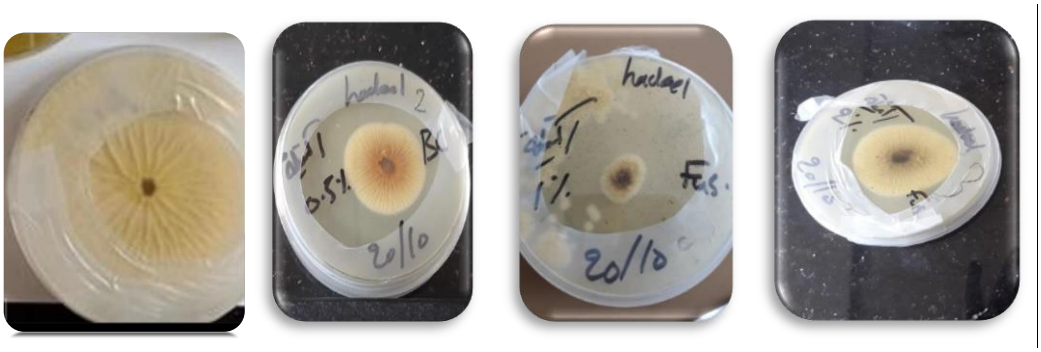


Figure (4.11): Effect of *V. iphionoide* extract on Mycelium Growth Rate *of F. oxysporum* at different concentrations (0%,0.5%,1% and 2%).

Results showed that *V. iphionoide* leaves extract had an antifungal effect by reducing the mycelial growth rate of *B. cinerea* and *F. oxysporum* pathogens. The leaves extract of *V.iphionoide* reduce the mycelium growth of *F. oxysporum* and *B. cinerea* at the concentration 0.5%-1 and at 2% gradually. The reduction of mycelium growth was positively correlated with increasing extract concentrations. The results of the effect of *V. iphionoide* leaves extract against *B. cinerea* and *F. oxysporum* were taken, and then MGR was calculated and illustrated by statistical analysis shown in table (4.3) .

Table (4.3): Statistical analysis of effect of *V. iphionoides* extract concentration on mycelial growth rate (R) against *B. cinerea* and *F. oxysporum.*

Fungi	**Concentration AS**				**Test statistic**	**p-value**
	0.0%	**0.5%**	**1%**	**2%**		
B. cinerea	9.74 ±0.00 a	4.42±1.11 ab	4.12±0.76 ab	2.35±1.26 b	9.596†	0.023**
F. oxysporum	13.76±0.00 a	3.66±1.62 b	2.83±1.83 b	2.41±2.13 b	5181.936‡	0.000**

†: Comparison of means using Independent samples kruskal wallis test.

- Different letters within row indicate a significant difference at the level 5%, the value represent means ± SD

- a and b, these letters indicate a statistically significant difference at a concentration of 0% and 0.5%

In table (4.3), there is a significant difference in MGR of *Botrytis cinerea* due to *Varthemia iphionoide* extract at 5% significant level. Also, MGR of *Botrytis cinerea* had the lowest value at 2% concentration which is (2.35±1.26 b). Independent samples kruskal Wallis test indicates there is a significant difference in the reduction of *Botrytis cinerea* between 2% concentration and 0% concentration. In addition, there is small differences in MGR of *Botrytis cinerea* between *Varthemia iphionoide* concentration (0.5%, 1%, 2%). The statistical analysis of MGR is illustrated by the graph in figure (4.12).

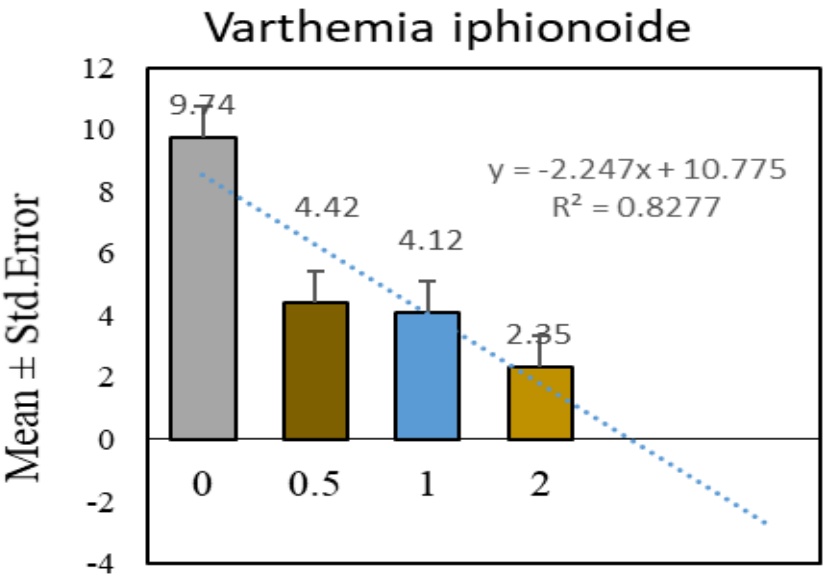


Figure (4.12) : Effect of *V.iphionoide* leaves extract at different concentrations on the Mycelium Growth Rate (R) of *B. cinerea*.

Figure (4.12) Indicates that there is negative relationship between MGR of *Botrytis cinerea* and *V. iphionoide* extract concentration, that's mean increasing of extract concentration of *V. iphionoide* reduce the growth of *Botrytis cinerea*. However increasing of *Varthemia iphionoide* concentration by 1%, reduce the growth of *Botrytis cinerea* by 82.77%.

Also, in table (4.3) show there is a significant difference in MGR of *Fusarium oxysporum* due to *Varthemia iphionoide* concentration at 0.5% significant level. Also, the rate of mycelium growth of *Fusarium oxysporum* had the lowest value at 2% concentration. Independent samples kruskal Wallis test indicates there is a significant difference of the rate of mycelium growth of *Fusarium oxysporum* between 2% concentration and 0% concentration. In addition, there is small differences of rate of reduction of *Fusarium oxysporum* between *Varthemia iphionoide* concentration (0.5%, 1%, 2%), The statistical analysis of MGR is illustrated in figure (4.13).

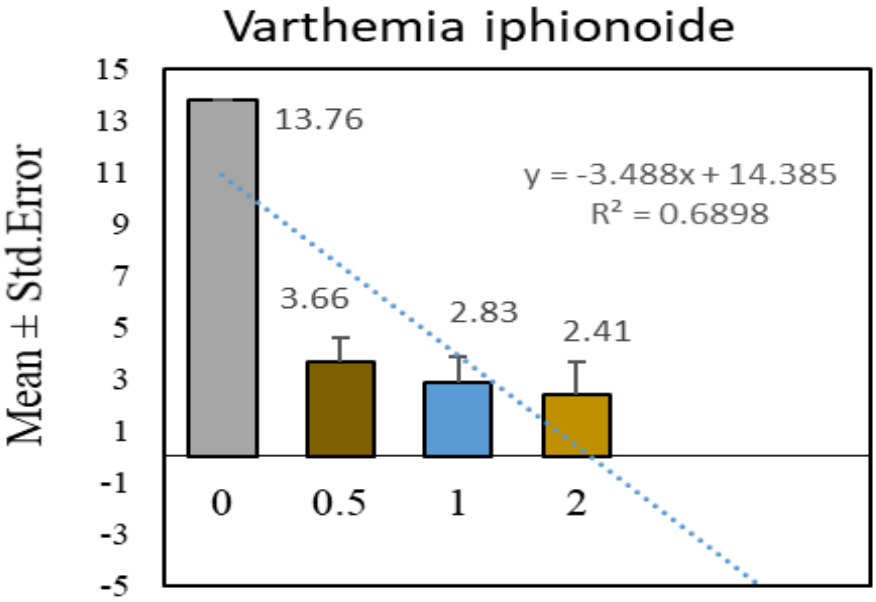


Figure (4.13): Effect of *V. iphionoide* leaves extract at different concentrations on the Mycelium Growth Rate (R) of *F. oxysporum* .

Figure (4.13) indicates that there is a negative relationship between MGR of *Fusarium oxysporum* and *V. iphionoide* extract concentration, which mean an increase in extract concentration of these medicinal plant reduce the growth *of Fusarium oxysporum*. In addition, increasing *Varthemia iphionoide* concentration by 1% reduces the growth of *Botrytis cinerea* by 68.98%.

4.3.1.3 Effect of *Achillea fragrantissima* leaves extract:

The effect of *A. fragrantissima* leaves extract on the mycelial growth rate of *B. cinerea* and *F. oxysporum* was evaluated in vitro by using a potatoes dextrose agar (PDA) medium. *A. fragrantissima* leaves extract, for each one of the three media samples were prepared with different concentrations, the volume of each sample was 100 ml, the results of *A. fragrantissima* on both *B. cinerea* and *F. oxysporum* is clear in figure 4.14 and 4.15.

Figure (4.14): Effect of *A. fragrantissima* extract on Mycelium Growth of *B. cinerea* at different concentrations (0%,0.5%,1% and 2%).

Figure (4.15): Effect of *A. fragrantissima* extract on Mycelium Growth of *F. oxysporum* at different concentrations (0%, 0.5%,1% and 2%).

Results showed that *A. fragrantissima* leaves extract had an antifungal effect by reducing of mycelial growth of *B. cinerea* and *F. oxysporum* pathogens. The leaves extract of *A. fragrantissima* reduce the mycelium growth *of F. oxysporum* and *B. cinerea* at the concentration 0.5%-1 and at 2% gradually. The reduction of mycelium growth was positively correlated with increasing extract concentrations.

Table (4.4): Statistical analysis of the effect of *A. fragrantissima* extract concentration on mycelial growth rate (R) against *B. cinerea* and *F. oxysporum*.

Fungi	**Concentration AS**				**Test statistic**	**p-value**
	0.0%	**0.5%**	**1%**	**2%**		
B. cinerea	9.74 ±0.00 a	4.59±0.28 ab	3.69±0.71 ab	2.26±0.07 b	10.569†	0.014**
F. oxysporum	13.76±0.00 a	4.40±0.44 ab	2.70±0.23 b	3.11±1.69 ab	8.556†	0.036**

†: Comparison of means using Independent samples kruskal wallis test.

- Different letters within row indicate a significant difference at the level 5%, the value represent means ± SD

In table (4.4), there is a significant difference in the MGR of *Botrytis cinerea* due to *Achillea fragrantissima* concentration at 0.5% significant level. Also, MGR of *Botrytis cinerea* had the lowest value at 2% concentration. Independent samples Kruskal Wallis test indicates there is a significant difference of MGR of *Botrytis cinerea* between 2% concentration and 0% concentration. The statistical analysis of MGR of *B.cinerea* is illustrated by the graph in figure (4.16).

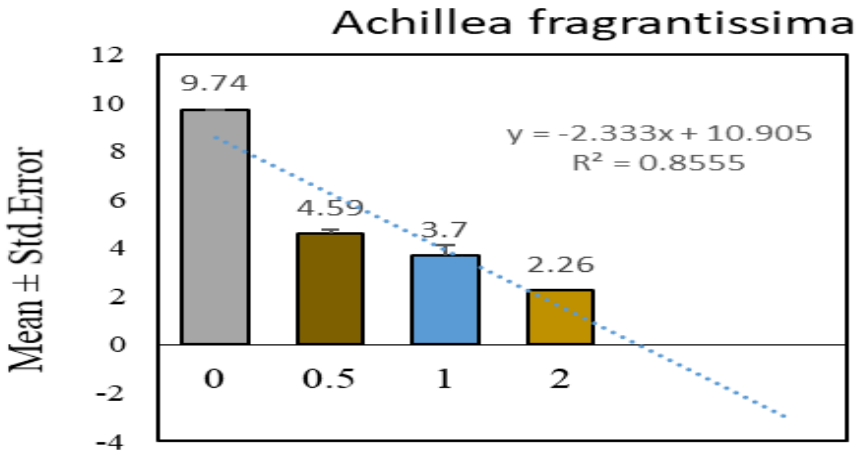


Figure (4.16): Effect of *A. fragrantissima* leaves extract at different concentrations on the Mycelium Growth Rate (R) of *B. cinerea*.

Figure (4.16), indicates that there is a negative relationship between the rate of reduction of MGR of *Botrytis cinerea* and *A. fragrantissima* extract concentration, that's mean increasing of extract concentration of *A. fragrantissima* reduce the growth of *Botrytis cinerea*. However, increasing *Achillea fragrantissima* concentration by 1% reduces the growth of *Botrytis cinerea* by 85.55%.

Results showed that there is a significant difference in MGR of *Fusarium oxysporum* due to *Achillea fragrantissima* concentration at a 5% significant level. Also, MGR of *Fusarium oxysporum* had the lowest value at 1% concentration. Independent samples Kruskal Wallis test indicates there is a significant difference of the rate of growth *Fusarium oxysporum* between 1% concentration and 0% concentration. In addition, there is small differences in the rate of growth of *Fusarium oxysporum* between *Achillea fragrantissima* concentration (0.5%, 1%, 2%). The statistical analysis of MGR of *B. cinerea* is illustrated by the graph in figure (4.17).

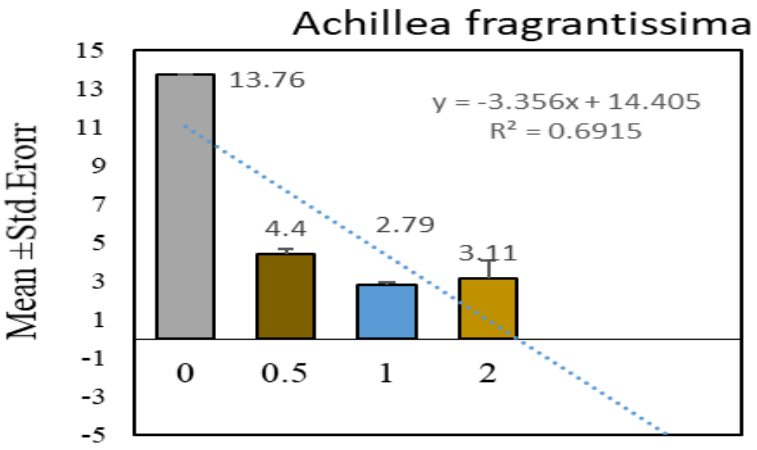


Figure (4.17): Effect of *A. fragrantissima* leaves extract in different concentrations on the Mycelium Growth Rate (R) against *F. oxysporum*

Figure (4.17) indicates that there is negative relationship between MGR of *Fusarium oxysporum* and *A. fragrantissima* extract concentration, which means an increase in extract concentration of *A. fragrantissima* reduce the growth of *Fusarium oxysporum*. Furthermore, increasing of *Achillea fragrantissima* concentration by 1%, reduce the growth of *Fusarium oxysporum* by 69.15 %.

4.3.1.4 Effect of Thymus persicus on MGR of two fungi:

The effect of *T. persicus* leaves extract on the mycelium growth rate of *B. cinerea* and *F. oxysporum* was evaluated in vitro by using potatoes dextrose agar (PDA) medium as shown in (Figure 4.13 and figure 4.14). Five media samples were prepared with different concentrations, the volume of each sample was 100 ml. the effect of *T. persicus* showed in Figures 4.18 and 4.19.

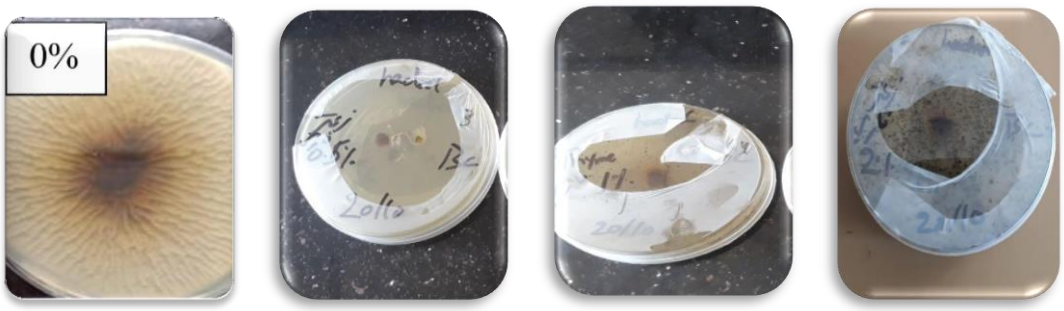


Figure 4.18: Effect *of T. persicus* extract on Mycelium Growth of *B. cinerea* at different concentrations (0%, 0.5%,1% and 2%).

Figure 4.19: Effect *of T. persicus* extract on Mycelium Growth of *F. oxysporum* at different concentrations (0%, 0.5%,1%, and 2%).

Results showed that *T. persicus* leaves extract had a strong antifungal effect on the mycelial growth rate of *B. cinerea* and *F. oxysporum* pathogens. The leaves extract at the concentration 0.5%,1% and 2% destroyed the mycelium growth of *F. oxysporum (*as shown in figures 4.19) and *B. cinerea (*as shown in figure 4.18). The reduction of mycelium growth was 100 % correlated with the presence of *T. persicus* extract concentrations. Results were illustrated in the table (4.5).

Table 4.5: Statistical analysis if the effect of *T. persicus* extract concentration on mycelial growth rate (R) against *B. cinerea* and *F. oxysporum*.

Fungi	**Concentration AS**				**Test statistic**	**p-value**
	0.0%	**0.5%**	**1%**	**2%**		
B. cinerea	9.74 ±0.01 a	0±0.00 b	0±0.00 b	0 ±0.00 b	11.00†	0.012**
F. oxysporum	13.76 ± 0.20a	0±0.00 b	0±0.00 b	0 ±0.00 b	11.00†	0.012**

†: Comparison of means using Independent samples kruskal wallis test.

- Different letters within row indicate a significant difference at the level 5%, the value represent means ± SD

- a and b, these letters indicate a statistically significant difference at a concentration of 0% and 0.5%

In table (4.5), there is no mycelium growth rate of *Botrytis cinerea* due to *T. persicus* extract at 0.5% ,1% and 2% concentrations. On the other hand, there is zero MGR of *Fusarium oxysporum* due to *T. persicus* consternation at 0.5%,1% and 2% significant levels. That's mean, the existence of *T. persicus* extract inhibited the growth of *Fusarium oxysporum*.

Discussion:

The medicinal plant extracts *S. dominica, V. iphionoide, A. fragrantissima* and *T. persicus* showed the strongest antifungal activity against *B. cinerea* and *F. oxysporum* in vitro by reducing the growth rate of mycelium; the activity increased with increasing concentration. The percentage reduction in mycelial growth ranged from 69% to 71% for *B. cinerea* and from 55% to 86% for *F. oxysporum*, depending on the concentration (0.5%, 1% and 2%). The highest growth area of both fungi was at 0.0% concentration (without addition of any concentration of extract), then it gradually started to decrease when extracts of *Salvia dominica* leaves were added at 0.5%, 1% and 2%. Furthermore, the percentage reduction in mycelial growth ranged from 54% -76% for *B. cinerea* and 73%-83% for *F. oxysporum*, depending on the concentration. The highest MGR was at zero concentration (without addition of extract), then it starts to decrease gradually

when *V. iphionoides* leaves extract was added at concentration of 0.5%, 1% and 2%. Moreover, the percentage reduction in mycelial growth ranged from (53% -77%) for *B. cinerea* and (68%-77%) for *F. oxysporum* depending on the concentration. *V. iphionoides* have dealt mainly with the characterization of its flavonoid constituents. A series of flavonoids, new for the genus, have been in fact isolated from the plant and their biological activity determined some of them showed antifungal effects (Sharawi and Barakat, 2009)

The highest MGR growth of both fungi was at 0.0% concentration (without extract), then it gradually began to decrease when extracts of *A. fragrantissima* leaves were added at concentrations of 0.5%, 1% and 2%, which has been shown in other studies that *A.fragrantissima* plays a role as an antifungal agent. *Achillea fragrantissima* essential oil showed significantly high antimicrobial activity against all tested microorganisms like cefaclor (5 mg/ml) and against fungi like fluconazole (10 mg/ml) in all model media. The chemical composition of essential oil of *A. fragrantissima* revealed that E.O contains many phytochemicals such as carvacrol which could be responsible for the antimicrobial activity of essential oil of *A. fragrantissima*. Therefore, it can be concluded that this essential oil can be used as a natural preservative for food. (Alsohaili and Al-fawwaz, 2014).

The essential oil of *A. fragrantissima* caused gross membrane damage and induced lysis of whole cells of eukaryotes. The mechanism of spread of antibiotic resistance from food animals to human's remains controversial and resistance to the same drugs. Most of the bacteria isolated from bovine mastitis showed multidrug resistance to antibiotics due to the presence of drug resistant genes such as beta-lactimase, which were easily detected and evaluated by PCR. This result is consistent with the explanation of the antibacterial activity of essential oil on bacterial cells, which depends on the action of the terpene fraction causing membrane disruption and marked leakage of cytoplasmic material, resulting in irreversible damage to the cytoplasmic membrane (Abdeen.et.al,2018).

In addition to the results of *T. persicus* which showed that the plant extract of *T. persicus* completely (100%) inhibited the mycelial growth of *B. cinerea* and *F. oxysporum* pathogene. This activity may be attributed to its essential oils which are used as antifungal agents in other studies. Carvacrol and thymol are two components present

not only in the essential oil of these aromatic plants but also in aqueous methanolic extracts. In a previous studies, HPLC analysis showed that the content of phenolic compounds in thyme is 41.07 mg/g, where carvacrol is the major phenolic compound in the dry aromatic plants (Skendi et.al.2017). The amount of phenolic compounds extracted from the dry matter in the aqueous solution was analyzed and related to the antifungal activity of the aromatic plant in PDA. Thyme efficiently inhibited 100% of fungal growth of Aspergillus and Penicillium in study of (Adriana Skendi et.al.2020). Thus, the reduction in the growth rate of mycelium is attributed to the aromatic compounds of the medicinal plants.

The antifungal activity may be attributed to the presence of different polyphenolic compounds and flavonoids such as those detected in plant extracts of the 4 plants analyzed in this study which includes the following compounds: 3,4-dihydroxybenzoic acid, kapmpferol, chlorogenic acid, quercetin, syringic acid, rutin, verbascoside, trans-cinnamic acid, sinapic acid, and p-coumaric acid. Structure of these compounds are shown in the following table (4.6). As shown from the structures of these compounds, the classes of these compounds are flavonoids-3 compounds (rutin, quercetin, and kampferol) and trans cinmaic acid, and the rest (6 compounds) are polyphenolic compounds.

Table 4.6.A: Polyphenolic compounds and flavonoids and its chemical structure.

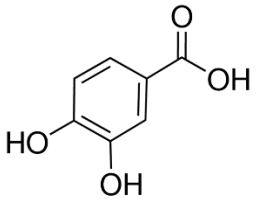
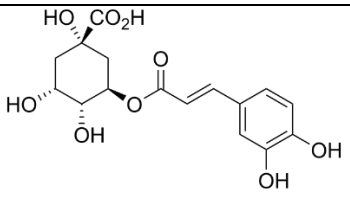
Standard name	Structure
3,4-Dihydroxybenzoic acid (Synonym: Protocatechuic acid)	
Chlorogenic acid	

Table 4.6.B: Polyphenolic compounds and flavonoids and its chemical structure.

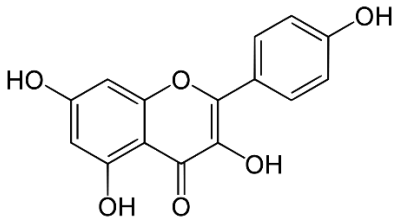
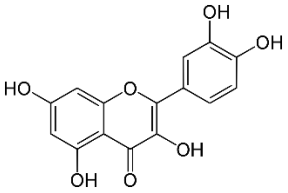
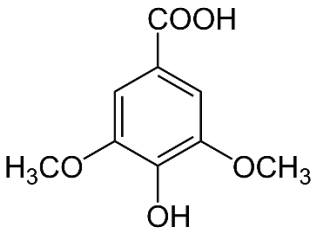
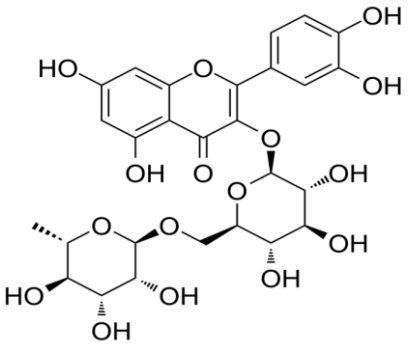
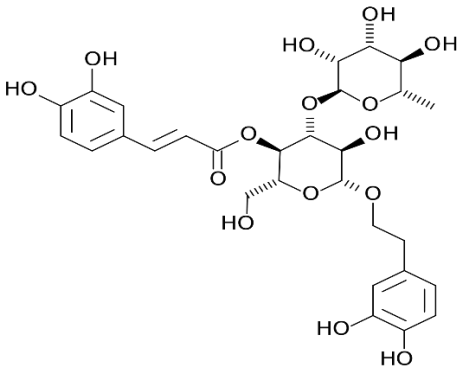
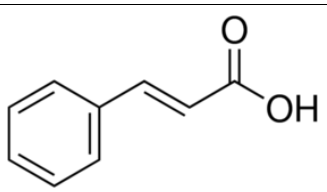
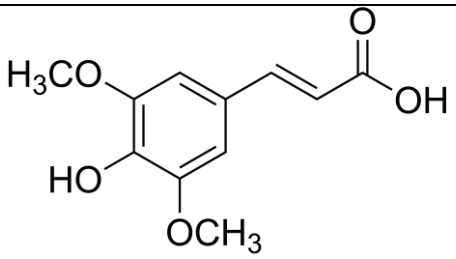
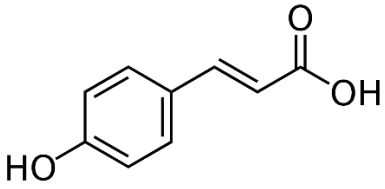
Kapmpferol	
Quercetin,	
Syringic acid	
Rutin	
Verbascoside	

Table 4.6.C: Polyphenolic compounds and flavonoids and its chemical structure.

Trans-cinnamic acid	
Sinapic acid	
p-coumaric acid	

Conclusion:

The study showed that some native Palestinian plants may be a potential source of antifungal compounds against *B. cinerea* and *F. oxysporum*. In vitro studies showed that the medicinal plants (*S. dominica, V. iphionoides, A. fragrantissima* and *T. persicus*) leaves extracts have high antifungal potential. The reduction in mycelial growth rate is correlated positively with the leaves extract concentration, although the degree of efficacy varied according to 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 is very important to increase the cultivated areas of these plants, especially thyme, in order to use them efficiently for the production of bio-pesticides. The integrated control study found that using a plant extract (*T. persicus*) in combination with a lower dose of commercial fungicides can help control gray mold disease. The plant extracts of *S. dominica, V. iphionoides, A. fragrantissima and T. persicus* analyzed using HPLC method showed the presence of different polyphenolic compounds and flavonoids such as: 3,4-dihydroxybenzoic acid, kapmpferol, chlorogenic acid, quercetin, syringic acid, rutin, verbascoside, trans-cinnamic acid, sinapic acid, and p-coumaric acid. These polyphenolic compounds would be responsible for the antifungal activity of these extracts.

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 the plants extracts on whole plants in greenhouses and field.
- Determine the formula of bio- fungicides for each plant extract to identify the individual active compounds.
- Further chemical analysis using GC-MS to specifically determine the bio active compounds in these nature products.
- In vitro and in vivo studies are still needed to detect the cyto toxicity of the plants extracts on whole plants.
- More field experiments, as well as more studies, are needed to corroborate and offer more light on the discovery of bioactive components in plants, mode of action, and stable formulations in the field.

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استخدام المبيدات الحيوية المستخلصة من نباتات فلسطينية برية للقضاء على بعض المسببات لأمراض المحاصيل

اسم الطالبة: هديل فريد عبد الحليم ارفاعية

المشرف: د. خالد صوالحة

الملخص:

يعد فطر البوترايتس المسبب الرئيسي لمرض العفن الرمادي، وهو أحد أكثر الامراض الفطرية المدمرة للنبات حيث أنه يصيب أكثر من 230 نوع نبات مختلف مثل الطماطم والعنب والفراولة وغيرها، مسبب عدة أمراض تؤدي الى خسائر اقتصادية في المحاصيل. بالإضافة الى فطر الفيوزاريوم الذي يعيش في التربة مسبباً عدة أمراض مثل الذبول والتعفن للسيقان والأبصال على أهم المحاصيل الاقتصادية مثل الطماطم والبطاطا والبقوليات والموز. كلا الفطرين يمكن السيطرة عليهم ومحاولة مكافحتهم بعدة طرق زراعية وكيميائية بهدف تقليل وجود الفطر وانتشاره. ولكن لسوء الحظ هذه الطرق أدت الى تطوير مقاومة العديد من المبيدات الفطريات الكيميائية التي لها آثار بيئية ونباتية وحيوانية وإنسانية سلبية. لذلك تم اقتراح العديد من البدائل بما في ذلك المكافحة البيولوجية والمستخلصات النباتية لتقليلها. في العقود الأخيرة تم التوجه للنباتات الطبية بما في ذلك نبات الخويخة ونبات الاكتيلة ونبات القيسوم ونبات الزعتر الفارسي كمصدر مهم لمركبات فعالة في صناعة الأدوية ومستحضرات التجميل وصناعة الأغذية والزراعة والمبيدات الطبيعية. لان هذه المركبات تعتبر آمنة، إضافة الى مستخلصاتها وزيوتها الأساسية النشطة بيولوجيا والتي أثبتت من خلال العديد من الدراسات أن لها تأثيرات مضادة للفطريات ومضادة للأكسدة. لذا كان الهدف من هذه الدراسة إيجاد بدائل للمبيدات الكيميائية المستخدمة بكثافة في فلسطين كمبيدات آمنة وعضوية وصديقة للبيئة من مستخلصات كل من نبات الخويخة ونبات الاكتيلة ونبات القيسوم ونبات الزعتر الفارسي من خلال فحص المضادات الفطرية الناتجة من كل من هذه النباتات ضد كل من فطر البوترايتس والفيوزاريوم. تم جمع أوراق الخويخة و الاكتيلة و القيسوم و الزعتر الفارسي في عام 2019 من بني نعيم ومن ثم تم تجفيفها وطحنها لاستخلاص اوراقها باستخدام الايثانول 99%. لدراسة التأثير البيولوجي لهذه المستخلصات تم إجراء تحليل HPLC باستخدام كاشف PDA لمزيج من المعايير والمستخلصات النباتية. تم فصل مزيج من مركبات البوليفينول والفلافونيدات باستخدام طريقة RP-HPLC. أظهر تحليل HPLC للمستخلص الإيثانولي لنبات الخويخة وجود 3،4-ثنائي هيدروكسي حمض البنزويك وكامبفيرول و حمض الكلوروجينيك و كيرسيتين و حمض سيرنجيك و روتين و فابكوسيديك. من ناحية أخرى ، أظهر المستخلص الإيثانولي لنبات القيسوم كل من مركبات روتين وحمض الكلوروجينيك وكامبفيرول وكيرسيتين ، وحمض ترانس سيناميك ، وحمض سينابيك ، وحمض ب-كوماريك وأيضا أظهر مستخلص نبات الزعتر الذي تم تحليله باستخدام طريقة RP-HPLC مركبات روتين و كيرسيتين و كامبفيرول ، بينما أظهر HPLC Chromatogram لمستخلص نبات الاكتيلة مركبات رويتن و كلوروجينيك اسيد و كامبفيرول . ومن ثم تم استخدام فحص يعمل على قياس معدل نمو الغزل الفطري الذي ينمو على الوسط الغذائي التي تحتوي على عدة تراكيز لكل مستخلص من النبات، من خلال هذه العملية تم الحصول على مستخلص من كل نبات. أظهرت

النتائج أن كلا من مستخلصات النباتات (الخويخة والاكتيلة والقيسوم والزعتر الفارسي)لها تأثير كبير على نمو الفطرين (البوترايتس و الفيوزاريوم) بحيث ان نمو الفطر يقل كلما زاد تركيز كل من المستخلصات مقارنة مع العينة التي لم يضف عليها أي مستخلص من النبات، حيث أظهر مستخلص الخويخة أثر على نمو الغزل الفطري لكلا من الفطرين (البوترايتس والفيوزاريوم) وكذلك الامر بالنسبة لنبات الاكتيلة والقيسوم ،حيث أنه بعد التحليل الاحصائي ظهر أن هناك اختلاف معياري في معدل انخفاض نمو كلا من البوترايتس والفيوزاريوم أي انه كل ما زاد تركيز المستخلص زاد معدل انخفاض نمو الفطر، لكن بالنسبة لعينات مستخلص نبات الزعتر الفارسي أظهرت النتائج بأنه قام بتدمير نمو كل من البوترايتس والفيوزاريوم حيث ان التثبيط وصل الى 100%.

توصلت هذه الدراسة إلى إمكانيات فعالة لبعض المستخلصات النباتية الطبية المختارة في السيطرة على البوترايتس والفيوزاريوم خاصة عندما تقترن بجرعة مخفضة من مركب مبيد للفطريات مما يعطي طريقة التحكم هذه بعدًا متكاملاً يمكن أن يساعد في تقليل المخاطر البيئية والصحية.

ومع ذلك هناك حاجة إلى مزيد من الدراسات في المجال وطريقة العمل والتركيب المستقر لهذه المستخلصات النباتية الطبية قبل الوصول إلى مرحلة التطبيق والاستنتاجات على نطاق واسع في مجال مكافحة مرض العفن الرمادي.