

## V. Discussion

Fungal diseases of plant and crops become a problem, especially during the wet weather season and need to be controlled to maintain the quality and abundance of food, feed, and fiber produced by growers around the world. *Penicillium* and *Aspergillus* species have been reported before as pathogens of fruit spoilage (219-223) specifically *Penicillium chrysogenum*, *Penicillium commune*, *Aspergillus fumigates* *Aspergillus flavus* and *Aspergillus oryzae*. *Penicillium chrysogenum*, previously known as *Penicillium notatum* can be found on salted food products and in damp buildings (224). *Aspergillus flavus* is a mycotoxigenic fungus infecting several crops including maize, moldy groundnuts, soyabean and other stored products [225-227]. Seedlings damping-off caused by *Rhizoctonia solani* and Fusarium wilt caused by *Fusarium Spp.* are regarded as the most important diseases that affect the tomato crop causing serious economic losses to the producers (228, 229).

A variety of biological controls are available for use, but further development and effective adoption will require a greater understanding of the complex interactions among plants, people, and the environment. The control of seedlings damping-off diseases of tomato is mainly based on the application of chemical fungicides, crop rotation and the use of pathogen-resistant varieties. However, fungicide application has resulted in the accumulation of residual toxicity in soil and vegetables, increase environmental pollution and alter the biological balance in the soil by decimating non-target and beneficial microorganisms. Adverse effects of chemical fungicides on the environment and human health are burning issues and there is a need to search for a new fungicides eco-friendly in nature.

Traditionally, microscopic and cultural techniques are often available for detecting and enumerating fungal spores. However, the methods are time-consuming and laborious. They require skilled and highly specialized expertise. Furthermore, microscopy is often fallible for identification of non-descript spores. Endophytic fungi are assessed by rolling the surface-sterilized plant tissue

samples, and subsequently isolation of fungal endophytes which grow in samples placed onto culture media (230, 231). However, cultural techniques are inappropriate for detection of slow growing or non-culturable fungi *in vitro* (232). Fungi that emerge from these samples can be identified by means of phenotypic (morphological) or genotypic (molecular) characters. In contrast, molecular techniques are sensitive and specific for identifying microorganisms. They can be used for the taxonomic classification and identification of microbial strains (233).

Molecular techniques have become the most powerful and indispensable tools in identification, community diversity, and phylogeny studies of endophytic fungi [234–238]. Also showed that identifying endophytes by blasting with GenBank sequences often resulted in the wrong naming of taxa and therefore any results must be treated with caution [239]. Thus, the reference sequences downloaded from GenBank database for blasting and conducting phylogenetic tree in this study were mostly published in previous studies [37–240, 241]. Among DNA markers, the ITS region is most commonly used for species delimitation. Recently, the ITS region has been confirmed to be applicable as a fungal barcode, and it is able to identify successfully a broad range of fungi (approximately 70%) [242]. Nine of the representative morphological isolates were further identified with molecular phylogenetic analysis of ITS1, ITS4 regions of the nuclear encoded rDNA showed significant alignments of 96–100% (Table 21, 22, Figure 13.1-13.4). According to the accession no. result in GenBank submission and phylogenetic analysis of studied plant pathogenic isolates were *F. oxysporum*, *F. solni*, *F. brachygibbosum*, *R. solani*, *A. fumigates*, *A. flavus*, *A. oryzae*, *P. chrysogenum*, *P. commune*. Generally, there was a good agreement between morphological and ITS-sequence based approaches.

Plants are rich source of bioactive compounds such as tannins, terpenoids, saponins, alkaloids, flavonoids, and other compounds, reported to have *in vitro* antifungal properties (243). The studied plants contained high amounts of bioactive compounds such

## THESIS

---

as total and simple phenolics, tannins, flavonoids, alkaloids, saponin and cyanogenic glycosides (37, 38). Therefore, in-vitro evaluation for antifungal activity of fifteen medicinal plant extracts were screened *in vitro* for antifungal activities assay against the mycelial growth of nine postharvest and phytopathogenic fungi, *F. oxysporum*, *F. solani*, *F. brachygibbosum*, *R. solani*, *A. Fumigatus*, *A. flavus*, *F.oryzae*, *P. chryogenum* and *P. commune* at concentration 4mg/well. The selected plants for antifungal evaluation were; *Rosmarinus officinalis*, *Zingiber officinale*, *Alium sativum*, *Alium cepa*, *Foeniculum vulgare*, *Nigella Sativa*, *Thymus vulgaris*, *Cassia Senna*, *mentha varidis*, *Syzygium aromaticum*, *Anethum graveolens*, *Eucalyptus globules*, *Lavandula angustifolia*, *Olea eurpaea* and *ziziphus spina-csisti*

The present study results showed that, the antifungal screening findings of fifteen herbal and medicinal plants essential oils, methanolic and aqueous extracts at concentration 4mg/well (40µl) against the selected nine fungal isolates according to the obtained data summarized in Tables (Table 23-31) revealed that The broad spectrum antifungal activity of the essential oils, methanolic and aqueous extracts of selected plants against phytopathogenic fungi has been demonstrate. The highest antifungal activity was exhibited by the essential oils that provided the greatest inhibition of mycelial growth and fungal spore germination spore germination of most of the fungal species tested. The oils capable of causing complete inhibition of spore germination of selected fungal species. While methanolic extracts of selected 15 plants were exhibit more efficient than the corresponding water extracts in most of antifungal activity assay according to the obtained data summarized in Tables (Table 23-31)

The present results are in accordance with the previous studies; Plant extracts obtained from various medicinal plants possess antimicrobial activity against many food borne, human and plant pathogens and pests (161, 244). Several studies have been conducted to check the antimicrobial properties of different herbs, spices, and the derivatives of these herbs and spices like essential oils, extracts and decoctions (77, 245 and 246). Many plant extracts obtained from medicinal plants have been used for food

preservation and for medicinal purposes due to their antimicrobial effects (152, 247-249). Some plants may be alternatives to currently used disease control agents since they constitute rich source of bioactive chemicals. Numbers of reports showed efficacy of essential oil of *Foeniculum* as antimicrobial agent. However, a few reports are found on in vitro antifungal activity of crude seed extract of fennel. In the present study, we have reported the effectiveness of essential oil, aqueous and methanolic extracts of *Foeniculum* against different spoilage fungi. Aqueous extract of *Foeniculum* fruits contains rich phenolic compounds, hydroxyl- cinnamic acid derivatives, flavonoid glycosides and flavonoids aglycones (250). The antifungal activity of *Foeniculum* extract may be due to presence of these secondary metabolites (251) and anethole (252). The mechanisms thought to be responsible for phytochemical toxicity of plant extracts to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more non-specific interactions with the proteins.

Our results revealed that the highest antifungal activity was recorded for *Foeniculum vulgare* oil, methanolic and aqueous extracts against *Fusarium oxysporum*, *Fusarium solni*, *Fusarium brachygibbosum*, *Rhizoctonia solani*, *Aspergillus fumigates*, *Penicillium chrysogenum*, *Penicillium adametzii* and *Aspergillus oryzae*, *Penicillium chryogenum* and *Penicillium commune* when compared with the control while low antifungal activity was recorded for *Aspergillus flavus* and *Aspergillus oryzae*. The obtained result is in accordance with that achieved by (253). The effectiveness of aqueous extract of *Foeniculum vulgare* in inhibiting the tested fungal species could be attributed to the fact that water is a polar solvent and the phytochemical constituents of *Foeniculum vulgare* such as flavonoids are very soluble in it hence retaining most of the antimicrobial (antifungal) properties. *Foeniculum vulgare* extracts have been reported to inhibit the growth of *Curvularia lunata*, *Fusarium oxysporum* and *Alternaria alternate* by (254). The alcoholic based extract was found adequately effective against *Mucor roxuii* but was completely ineffective against *Aspergillus flavus* and *Alternaria alternata*.

In consistent with the present result Soylu EM, et al., 2006 (255) Various plant extracts have been reported to exert different levels of antifungal activity in vitro against

## THESIS

---

phytopathogenic fungi, including thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), fennel (*Foeniculum vulgare*) and *Phytophthora infestans*

The present result of high antifungal activity of *Foeniculum vulgare* oil compared with corresponding methanolic and aqueous extracts is in agreement with previous study of Gurdip Singha, et al., 2006 (256). we can say that *F. Vulgare* volatile oil, which is rich in transanethole, possesses good antifungal activity against *A. niger*, *A. flavus*, *F. graminearum* and *F. moniliforme* whereas its acetone extract, which is rich in linoleic acid, can be used as natural antioxidant for linseed oil. Hence, they are valuable for increasing shelf life of foodstuffs and protector for highly unsaturated linseed oil, replacing synthetic fungicides and antioxidants such as BHT and BHA, as well as for preventing cellular damage, the cause of aging and human diseases. In addition the obtain results summarized in tables (23-31) in agreement with earlier studies revealed that various plant extracts have been reported to exert different levels of antifungal activity in vitro against phytopathogenic fungi, including thyme (*Thymbra spicata* subsp. *stoechas*), oregano (*Origanum syriacum* var. *bevanii*), rosemary (*Rosmarinus officinalis*), fennel (*Foeniculum vulgare*) and *Phytophthora infestans* [257]. Also, the leaf extract of Daruta metal has strong inhibitory effect against *R. solani* and *Xanthomonas oryzae* pv. *oryzae* under both in vitro and in vivo conditions. The antifungal nature is known to be due to the presence of daturilin, a withanolide compound isolated from Daruta metal [255, 582]. The extracts of *Pongamia glabra* inhibited the spore germination of *Alternaria solani* [259]. The maximum reduction in the mycelial growth of plants may be due to the presence of antifungal compounds in the extracts. A concentration of 10% zimmu leaf extract was highly effective in reducing the mycelial growth (by 87%) of *A. solani* [258]. In this result, methanolic extracts of TNB showed strong inhibitory activity against the growth of *F. solani* when investigated by direct contact with the extract using in vitro assays. The *F. solani* mycelia growth and mycelia hypha were affected by GA purified from TNB. One of the mechanisms by which compounds exert their antifungal activity is through the degradation of cell walls of pathogenic fungi. Such inhibitors are believed to block the synthesis of chitin in fungal cell walls [260]. In this regard, gallic acid may be

producing the enzyme chitinase that degrades chitin. Because of their ability to degrade chitin, the major structural component of the cell walls of phytopathogenic fungi [261], chitinolytic enzymes are considered important in the biological control of soil-borne pathogens [262].

From results presented and collected at table (23-31) shown high effectiveness of *Allium sativum* oil, methanolic and aqueous extracts for most of studied isolated fungi. These findings coincident with the study of Okigbobet al. 2009 [263] investigated the fungi toxic effects of *A. sativum* against six plant pathogenic fungi such as *F. oxysporum*, *F. solani*, *Botryodiplodia theobromae*, *M. phaseolina*, *Penicillium oxalicum* and *Aspergillus niger*. The obtained results revealed *A. sativum* had effective inhibition on the mycelial growth of all tested fungi. Asha Kaji Shrestha et al., 2009 (264) suggested that many extract of studied plants, showed inhibition effects on the growth of *Fusarium spp.* Among the tested plant species, *Allium sativum* markedly inhibited the *F.solani* fungus growth even at a low concentration

In addition the results shown in tables (23-31) indicate significant inhibition of germination of most of studied fungi. Previous study reported that, antifungal tests of *A. sativum* and *Z. officinalis* extracts exhibit antifungal activity against *F.spp.*, *Rhizoctonia solani*, *A. spp.* and *Penicillium spp.* (265, 266) were reported that higher concentration of antimicrobial substance showed appreciation in growth inhibition as compared to control.. All the oils tested exhibited different degrees of antifungal activity against toxigenic *A. flavus*. The maximum antimycotic activity was shown by *A. sativum* followed by *Z. officinalis*. (267). and the essential oils of, *C. limon* and *C. aurantifolia* exhibited moderate

Antifungal Assays results in tables (23-31) showed that *Thymus vulgaris* and *Z. officinale* extracts provided stronger inhibition of germination of most of the fungal species tested. Earlier study showed that *Thymus vulgaris* and *Z. officinale* extracts provided a significant inhibition of mycelial growth of the phytopathogenic fungi and their sensitivity to a given plant extract varied greatly.

These results are in accordance with that of Ramanathan et al. 2004 (268) and Jung et al. 2003 (269). *Thymus vulgaris* extract showed complete suppression on colony growth of *Fusarium oxysporium*, *Pythium aphanidermatum* and *R. solani* followed by extract of *Z. officinale* which appear to be effective against *Pythium aphanidermatum* and *Rhizoctonia solani* and less effective against *Fusarium oxysporium*. In addition the antifungal activity of *Thymus vulgaris* essential oils has well proved against fungi such as *Aspergillus* spp. (270), *Rhizoctonia solani*, *Fusarium solani* (271). Thyme essential oils in addition to complete inhibition growth of grain storage fungi, prevents toxin production (272). . In addition accordance with that Al-Rahmah et al., 2013 (273) two methanolic extracts were screened in vitro to evaluate them to control tomato phytopathogenic fungi (*Pythium aphanidermatum*, *Rhizoctonia solani* and *Fusarium oxysporium*). Assays showed that, three plant extract provided a significant inhibition of mycelial growth of the phytopathogenic fungi and their sensitivity to a given plant extract varied greatly. *Thymus vulgaris* extract showed complete suppression on colony growth of *Fusarium oxysporium*, *Pythium aphanidermatum* and *Rhizoctonia solani* followed by extract of *Z. officinale* which appear to be effective against *Pythium aphanidermatum* and *Rhizoctonia solani* and less effective against *Fusarium oxysporium*.

According to, the obtained data summarized in tables (23-31) revealed that *Syzygium aromaticum* clove provided the greatest inhibition of spore germination of most of the fungal species tested. Earlier study of *Syzygium aromaticum* and *A. sativum* were successfully effective in suppressing the *Fusarium* growth *in vitro* (274). The antifungal activity of the clove oil and its main component neugenol [275], were investigated against *Candida*, *Aspergillus* and dermatophyte clinical and American Type Culture Collection strains [276] which showed inhibitory activity against all the tested strains. Mainly, phenolics components of essential oils are considered as responsible for the antimicrobial activity, followed by aldehydes, ketones, and alcohols [39, 277-278].

## THESIS

---

Several workers (279, 280) have earlier reported that clove ethanolic extract showed antimycotic activity against fungal genera such as *Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium* and *Saccharomyces* which is in harmony with the present study. This activity may be due to the presence of eugenol and caryophyllene

Antifungal assay of *Allium* vegetable extracts (*Allium sativum* and *A. cepa*) extracts were very effective in inhibiting the growth of *Aspergillus* species. Our findings in agreement with Ozer et al. 2003 (281) evaluated the pectolytic impact of *Allium cepa* “Akugun 12” against two *Fusarium* isolates FOC6 and FOC8. In addition *A. Sativum* was known to act as anti-fungal activity (282). Similar results were found by (283) and (245) using *Allium sativum* against eighteen different fungi including *Fusarium* spp. also Essential oils from *Allium sativum* and 3 other plants were promising inhibitors of *Rhizoctonia solani* causing sheath blight of rice (284). The inhibitory activity of *Allium* vegetable extracts against molds have been reported by numerous authors (285, 286). Allicin, thiosulfonate and other compounds showed fungistatic activity against *Aspergillus* spp. such as *A. flavus*, *A. fumigatus*, *A. terreus* and *P. chrysogenum* (287).

In this study selected essential oils, methanolic and aqueous extracts of *Mentha varidis* were screened for antifungal activity against selected phytopathogenic fungi. The result shows different levels of antifungal activities. *Mentha varidis* oils showed excellent antifungal activity against the selected fungi Followed methanolic with more activity than corresponding aqueous extracts and Our results in accordance with that (288). The antimicrobial effects of essential oil taken from spearmint plants for *F. oxysporum* fungus depend on the kind of compound and its chemical components. More than twenty kinds of chemical compounds have been reported from essential oil extracted from spearmint. The most important one with antimicrobial activity are Menthol, Menthoforan, Menthon, Pipriton and Polgon. [289] Essential oil of Cinnamon, Peppermint, Basil, Origanum, Clove and thyme were proved to cause total inhibition of *A. flavus* on maize kernels. [290] Eucalyptus oil and clove oil showed activity against *F. solani* *A. oryzae*, *A. flavus* and *A. Fumigatus*. In addition earlier report of Luma Taha Ahmed et al., 200 [291]

## THESIS

---

Demonstrate the inhibition effect obviously of Eucalyptus extracts on the growth and the average diameter of *A. flavus* & *Penicillium* sp. as compared with control group of both fungi.

The present results of antifungal assay of *Anethum graveolens* oil, methanolic and aqueous extracts against studied fungi and summarized in tables (23-31) revealed different level of inhibition of spore germination for selected fungal species. A few studies of Soylu, et al., 2006 and El gayyer, et al., 2001 [255, 267] have been reported the anticandidal effect of essential oil of dill seeds). The dill extracts were consistently found to be effective on fungal growth by inhibition of sporangial production.

The minimum inhibitory concentration result of the antifungal assay of oils, methanolic and aqueous extracts of selected fifteen plants by broth dilution technique summarized against nine plant pathogenic fungi at table (32) all tested plant extracts were showed antifungal activity with MIC values ranging from 0.25 to 4mg/ml. The tested extracts showed different levels of antifungal activity depending on tested species. Finally, the finding of the present investigation could be an important step towards the possibilities of using natural plant products as biopesticides in the control of plant diseases

The results from the present study indicated that the Qualitative phytochemical screening result of the crude extracts of fifteen selected medicinal plants for secondary metabolites shows summarized in table (33) confirmed the presence of carbohydrate, protein, phenols, saponins, tannins, terpenoid, flavonoids and volatile oils qualitative phytochemical screening results revealed that, proteins, carbohydrates, were present in all the selected fifteen plant extracts except *mentha varidis* and *Anethum graveolens* , alkaloids were absent in five extracts; *Nigella Sativa*, *Thymus vulgaries*, *Lavandula angustifolia*, *Olea eurpaea* and *ziziphus spina-csisti*. However, preliminary phytochemical screening showed that, the presence of Flavonoids in all samples except *Lavandula angustifolia*. And the Tannins present in all study samples except *Olea eurpaea*. And the Terpenoids were found with different levels in all the selected extracts

except in *Eucalyptus globules*. Finally, saponins compounds were found in all the study samples except *Lavandula angustifolia* and *Olea eurpaea*. The phenolics compounds present in most of samples except only 6 extracts; *Alium sativum*, *Alium cepa*, *Foeniculum vulgare*, *Anethum graveolens*, *Lavandula angustifolia* and *ziziphus spina-csisti*. Several workers investigated the preliminary phytochemistry of medicinal plants Krishnaiah et al., 2009; Koche et al., 2010 (292, 293). Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries of the world and are good source of many potent and powerful drugs (294). In Africa, the use of plant derived remedies in traditional health care practices is common and widespread (295) even before the introduction of synthetic antibiotics and other modern drugs (296).

These results of our study support the work of Christy Jeyaseelan et al., [297]. They reported that the fresh leaf aqueous extracts of *Seena alata* showed the presence of glycosides, alkaloids, saponins, tannins, flavonoids, terpenoids and anthraquinones. The difference in the above results in the respective solvents may be due to the interaction of the phytoconstituents with the solvent system or the process employed for extraction. Other earlier studies showed that the power of these Phytochemicals compounds to exert higher activity is depending to their concentrations in the extracts (298). Among the Phytochemicals compounds with antifungal activity, mainly cites alkaloids, polyphenols and steroids (398, 299). The antifungal activity of the extracts of the plant depends on its composition, the plant organ to be tested, the nature of the extract and the fungal strains selected (300, 301).

Several studies have been conducted to understand the mechanism action of plant extracts. Many researchers attribute this feature to phenolic compounds. Phenols are one of the largest and most ubiquitous groups of plant metabolites. A number of biological properties such as antiapoptosis, antiageing, anticarcinogen, anti-inflammation and cell proliferating activities are attributed with phenolics [302]. These compounds can interfere with biomembranes causing cell damage and causing leakage of cellular materials and finally the death of microorganisms (300, 303). This is a possible mechanism by which

the mycelial growth can be reduced or completely inhibited by the effect of extracts acting on the function and structure of the cell membrane. Saponins are a special class of glycosides with a soapy characteristic and very good antifungal activity (304).

In earlier literature, many reports revealed that Flavonoids are widely distributed in plants. They are known to be responsible for the yellow or red/blue pigmentations in flowers and also provide protection from attack by microorganisms and insects (305). They are also responsible for the inhibition of resistant microbes. They are responsible for the scavenging process or chelators and may disrupt microbial membranes. Furthermore, Alkaloids are a group of naturally occurring chemical compounds and chief class of plant secondary metabolites. They are bitter to taste and are toxic to other organisms [306] and hence act in inhibiting microbial growth. The antibacterial and antifungal properties of the plant may be due to the presence of alkaloids. Alkaloids contain a detoxifying effect and have a very good antifungal activity (307). Terpens (steroids) affect not only the permeability but also other functions in cell membranes. These compounds can penetrate cell membranes, enter the interior of the cell, and interact with critical sites such as intracellular enzymes and proteins, leading to cell death (112). On the other hand, Antifungal activities have been reported for several kinds of saponins (308). Saponins appear to act by disrupting the membrane integrity of fungal cells (243).

Phytochemical constituents are secondary metabolites of plants that serve a defense mechanism against predation by many microorganisms, insects and other herbivores (309). The mode of action of these Phytochemicals may be via lysing the cell, increasing permeability of the cell wall and membrane, inhibition of protein and DNA synthesis and or by inhibiting the transport of nutrients across the cell wall or membrane (310). This inhibitory effect of the extract on the growth of these microorganisms could be attributed to the presence of some Phytochemicals that were found present in the plant extract. Saponins have detergent properties and serve as lytic agents and exhibit anti-inflammatory properties (311) while alkaloids and glycosides with the aid of their defense mechanism act as phytoprotective agent against invading microorganism. The demonstration of antibacterial activity against both gram positive and gram negative

bacteria by the plant may be indicative of the presence of broad spectrum antibiotic compounds (312).

In this study, the presence of phytoconstituents in the methanolic extracts of selected medicinal plants was further confirmed by thin layer chromatography using the solvent system, CHCl<sub>3</sub>: MeOH in a 4:1 ratio visualized under UV 254 nm. This qualitative techniques will give an idea of the presence or absence of bioactive compounds which shown as coloured spots in the different separated extracts. The result of TLC chromatographic separation Fig (32.1, 32.2) revealed that find reddish brown active spots in TLC with different R<sub>f</sub> values in three samples (*Cassia Senna*, *mentha varidis* and *Anethum graveolens*); these are indicated that the three samples rich in alkaloids compoumnds. And presence of spots ranged from dark to light yellow with different R<sub>f</sub> values in five samples (*Thymus vulgaries*, *Cassia Senna*, *mentha varidis*, *Anethum graveolens* and *Olea eurpaea*); these are indicated that the five samples rich in flavonoids & flavones compoumnds. Previously it was reported that *N. sativa* seeds contain tannins, which is extracted in methanolic extract (313). Tannins forms complexes with proteins through forces such as hydrophobic effects, hydrogen bonding and covalent bond formation, thus, tannins act as antibacterial agent by inactivating microbial adhesions, enzymes, cell envelope transport proteins (314).

The extracts with potential antifungal activity essential oils of selected medicinal plants were subjected for the GC-MS Analysis. The obtained results reveal that Presence of tested secondary metabolites summarized in Tables (34-48). In conclusion the major separatable Phytochemicals compounds present in essential oils of fifteen have a quite number of, which have many pharmacological activities responsible for the antifungal activity of these plants such as: Eucalyptol, Limonene, D-Limonene , Linalool, Zingiberene, thymol , p-cymene, Eugenol, Alpha- Farnesene, Carveol, propyl trisulphide, methyl propyl trisulphide, Menthol, Estragole, Octadecadienoic acid, n-Hexadecanoic acid, methyl ether, Alpha- Phellandrene, Menthone, Alpha- Terpir,

Gamma- Terpineol, piperitone, Palmitic acid, Lavandulyl acetate, Caryophyllene, Oleuropein, Verbascoside, Geranyl acetone and Methyl hexadecanoate. The difference in composition found on the essential oils investigated is likely to be related to abiotic factors such as climate-specific regions of origin of samples, geographical factors such as altitude and soil type. Earlier literature indicated that medicinal plants are the back bone of traditional medicine and the antibacterial activity of plant extract is due to different chemical agent in the extract, which was classified as active antimicrobial compounds (315).

On the other hand, In agreement with our study chemical analysis performed by GC/MS revealed that essential oil of *Allium sativum* is characterized by the presence of major compounds, which are: methyl allyl trisulfide (34.61%) and diallyl disulfide (31.65%) together with other constituents at relatively low levels (316). In another study, Allicin (diallyl-dithiosulfinate), which is produced by the garlic enzyme alliinase from the alliin, has been shown to have wide-range antifungal specificity. An in vivo study showed that antibody-alliinase conjugates and alliin are effective against murine pulmonary aspergillosis (317). One study showed that allicin from garlic has antifungal activity particularly against *Candida albicans* (10). Another in vitro study showed both intrinsic antifungal activity of allicin and its synergy with the azoles, in the treatment of candidiasis (318). Studies on the effect of Amphotericin B (AmB) against *C. albicans* showed that allicin enhances significantly the effect of AmB against *Candida albicans*, *Saccharomyces cerevisiae* and against *Aspergillus fumigatus* in vitro and in vivo (319, 320). It was found in another study that polymyxin B (PMB), is effective against various yeasts and filamentous fungi when used in combination with allicin. This combination increases the plasma membrane permeability in *Saccharo cerevisiae*. Swollen spherical structure of the yeast disappeared as a result of structural alterations of its vacuole caused by the synergistic activity between PMB and allicin combination (321). It has been reported in earlier observations The major compounds of the *Rosmarinus officinalis* oil were camphor (26.40%), 1, 8-cineole (23.40%),  $\alpha$ -pinene (9.94%), camphene (5.83%), myrcene (4.86%), bornyl acetate (3.97%), verbenone (3.32%), limonene (3.08%),

borneol (2.05%) and  $\alpha$ -terpineol (2.68%) (322). However the major chemical compositions of *Z. officinalis* oils are present in the organic ginger oil were camphene (4%), sabinene (3%), neral (2.7%) and geraniol (1.8%). The total oxygenated compounds found present in the organic ginger oil was 35.1% and the hydrocarbon content was 63.9%. The major oxygenated compound was geraniol (10.5%) followed by 1,8 cineole (3.5%), neral (2.7%), borneol (2.3%),  $\alpha$ -terpineol (2.1%). Similarly main sesquiterpene compound was zingiberene (323). The essential oils of summer *Thymus vulgaris* was characterized by phenol compound like thymol which was in agreement with the results of previous studies of these oil (324, 325). Thymol was predominant component of thyme oil (43.7%). Also, these three oils contained a significant proportion of biological precursors of phenolic components, terpinene and p-cymene. Rose and pelargonium oil were found to be characterized by two major compounds, monoterpene alcohols, citronellol and geraniol, as well as in other studies (326). In other research investigation Identified compounds in *Thymus longicaulis* show that Main constituents were  $\alpha$ -terpineol (14.23%),  $\alpha$ -terpinyl acetate (11.81%), linalool (9.63%), and myrcene (6.59%). In L3 sample 88.08% of the compounds were identified with thirty-three constituents resulted in total. Main compounds were monoterpene hydrocarbons (41.70%), oxygenated monoterpenes (28.17%), and sesquiterpene hydrocarbons (14.64%). Main constituents were  $\alpha$ -phellandrene (19.07%), linalool (14.22%),  $\beta$ -caryophyllene (9.27%), limonene (7.83%), and camphene (7.26%) (327)

On comparison of the present results of chemical composition of *Lavandula angustifolia* oil with previous reports (328, 329) The major compounds of the those reported from samples of other countries, it is quite evident that the concentrations of 1,8-cineole, camphor, (Z)- $\beta$ -ocimene, (E)- $\beta$ -ocimene, hotrienol and borneol were slightly higher, whereas the concentration of nerol, caryophyllene, elemol, cadinene, terpineol, thymol acetate, lavandulol, limonene and  $\alpha$ -pinene were marked less in this sample. Essential oils of lavender characterized by monoterpene components: linalool

(monoterpene alcohol), limonene (monoterpene hydrocarbons) and linalool acetate (monoterpene acetate) (330).

Earlier report of Saptha J. et al., 2009 (331) The GC-MS analysis of the *Nigella Sativa* volatile oil showed the presence of biological active compounds such as  $\alpha$ -thujene, 2(1H)-naphthalenone,  $\alpha$ -pinene,  $\alpha$ -phellandrene, limonene, thymoquinone, myristicin etc in *N.sativa* volatile oil contributed the antimicrobial activity of volatile oil). Another research reported that nigella seed oil contained significant amounts of sterols. Linoleic (C18:2), oleic (C18:1) and palmitic (C16:0), as in most of the common edible oils, are the main fatty acids [332]. On the other hand, Our result In agreement with Thymol is present in the methanol extract of *N. sativa* was investigated by (333) that thymol is responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through nonspecific interactions with the proteins.

It has been well established fact (from earlier and the present study) that eugenol is that most abundant component of *Syzygium aromaticum* oil, respectively Other major constituents of oils were trans- ( $\beta$ )-caryophyllene . It is noteworthy that eugenol has many clinical implications as good local antioxidant, antiseptic, local anaesthetic and analgesic (334). Our results were in agreement with the results of (335) according to which the methyl chavicol was the main component in basil oil. Lemon, orange and eucalyptus essential oils were the most similar in the chemical composition, with high concentration of monoterpene alcohol, limonene. Limonene content was as high as 90% in orange and eucalyptus oil and up to 65, 9% in lemon oil. Our analysis gave a similar result (336) reported flavour components analysed by GC-MS in a commercial *Allium cepa* oil. The most abundant compound was propyl trisulphide (23.07%) followed by propyl disulphide (22.48%), methyl propyl trisulphide (14.88%), 1-propenyl propyl disulphide (7.24%), propyl tetrasulphide (6.55%) and methyl propyl disulphide (2.81%).

Finally, the findings are also in line of previous findings and reported literatures. However the observed differences in the concentration of chemical constituents of essential oils from studied plants may be due to different environmental and genetic factors, different chemotypes and the nutritional status of the plants. The finding of the

## THESIS

---

present investigation is an important step towards isolation and characterization of the antifungal agent and its further evaluation for crop protection strategies. The studied plants being an edible plant (non toxic to human and easily biodegradable) possessing significant broad spectrum antifungal activity against important field and storage fungi would probably be an important candidate plant for prevention of biodeterioration of grains during storage and prevention of spoilage of processed food product.