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M.Sc. in Food Engineering

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REPUBLIC OF TURKEY

GAZİANTEP UNIVERSITY

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ANTIMICROBIAL PROPERTIES OF AROMATIC PLANTS COLLECTED FROM TURKEY AND SYRIA

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Supervisor

Prof. Dr. Osman ERKMEN

By

Anas ALMOHAMMAD

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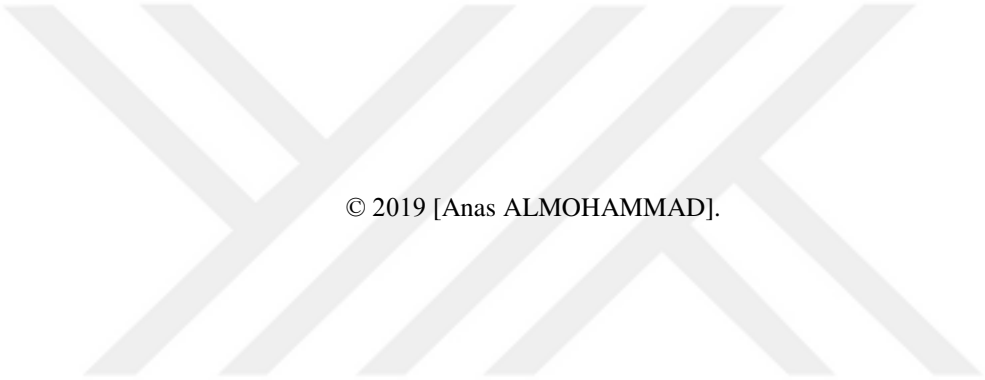

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Anas ALMOHAMMAD

ABSTRACT

ANTIMICROBIAL PROPERTIES OF AROMATIC PLANTS COLLECTED FROM TURKEY AND SYRIA

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This study was carried out to determine the antimicrobial properties of aromatic plant essential oils; thyme, anise, mint, chamomile, and basil were collected from Gaziantep in Turkey and from Aleppo and Idleb in Syria. The essential oils were assayed against following microorganisms: *Escherichia coli, Salmonella enterica* subsp. *enterica* ser. Typhimurium, *Yersinia enterocolitica, Pseudomonas aeroginosa, Listeria monocytogenes, Shigella dysanteria, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Klebsiella pneumoniae, Enterococcus faecalis, Streptococcus thermophilus, Saccharomyces cerevisiae, Candida rugosa, Aspergillus flavus, Aspergillus ochraceus, Aspergillus niger, Penicillium expansum, Rhizopus oryzae* and *Fusarium graminearum*.. The MIC (minimal inhibitory concentration), MBC (minimal bactericidal concentration), and MTC (maximum tolerance concentration) were determined for these five types essential oils against the microorganisms. The MIC values for thyme essential oils ranged from 0.1 to 1.25 µg/ml while the MBC from 0.5 to 1.75 µg/ml. For anise essential oil MIC values varied from 5 to 70 µg/mL while the MBC from 10 to 80 µg/ml. For mint essential oils the MIC ranged from 10 to 90 µg/mL while the MBC from 20 to 100 µg/mL. For chamomile essential oils the MIC ranged from 2.5 to 125 µg/mL while the MBC from 2.5 to 130 µg/ml. For basil essential oils the MICranged from 7.5 to 60 µg/mL while the MBC from 10 to 75 µg/ml. The MIC/MBC ratio for essential oils obtained from Turkey and Syria plants is closed to 1. On the other hands, the Turkish and Syrian essential oils have a more antimicrobial effect on selected microorganisms than other species essential and the Syrian basil has higher MIC means than essential oils of species that extracted from the neighbor cities (Gaziantep in Turkey and Aleppo and Idleb in Syria) have similar compounds so, they have similar antimicrobial effect.

Key Words: Aromatic plants, essential oils, MIC, MBC, MTC.

ÖZET

TÜRKİYE VE SURİYE'DEN TOPLANAN AROMATİK BİTKİLERİN ANTİMİKROBİYAL ÖZELLİKLERİ

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Bu çalışma, Gaziantep'ten (Türkiye) ve Halep, İdleb'ten (Suriye) toplanan kekik, anason, nane, papatya ve fesleğen gibi aromatik bitkilerin antimikrobiyal özelliklerini belirlemek için yapılmıştır. Uçucu yağlar, şu mikroorganizmalara karşı test edildi: *Escherichia coli, Salmonella enterica subsp. Enterica ser. Typhimurium, Yersinia enterocolitica, Pseudomonas aeroginosa, Listeria monocytogenes, Shigella dysanteriae, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Klebsiella pneumoniae, Enterococcus faecalis, Streptococcus thermophilus, Saccharomyces cerevisiae, C.rugosa Aspergillus flavus, Aspergillus ochraceus, Aspergillus niger, Penicillium expansum, Rhizopus oryzae ve Fusarium graminearum*. Tüm mikroorganizmalar Amerikan tipi kültür koleksiyonundan elde edildi. Mikroorganizmalara karşı bu beş bitki esansiyel yağı için MIC (asgari inhibitör konsantrasyonu) ve MBC (asgari bakterisit konsantrasyon) belirlendi. Kekik esansiyel yağları için MIC değerleri 0.1 ile 1.25 µg/ml arasında değişirken, MBC 0.5 ile 1.75 µg/ml arasında değişmiştir. Pimpernel esansiyel yağı için MIC değerleri 5 ile70µg /ml, MBC ise 10 ile80µg/ml arasında değişmiştir. Folium esansiyel yağları için MIC10 ile90µg/ml arasında değişirken, MBC 20 ile 100µg/ml arasında değişmütir. Papatya esansiyel yağları için MIC 2.5 ile 125 µg/ml arasında değişirken, MBC 2.5 ile130µg /ml arasında değişmıştir. Fesleğen esansiyel yağları için MIC 7.5 ile 60 µg/ml arasında değişirken, MBC10 ile75µg /ml arasında değişmiştir. Türkiye ve Suriye'de ki bitkilerinden elde edilen uçucu yağlar için MIC/MMC oranı 1,0. Diğer taraftan Türkiye' den ve Suriye' den getirilen aromatik bitkiler, seçilen mikroorganızmalar üzerinde diğer türlerden daha fazla antimikrobiyal etkiye sahiptir. Suriye fesleğeni diğerlerine göre daha yüksek MIC ortalamasına sahiptir. Türkiye' dekiGaziantep gibi komşu şehirlerden ve Suriye'deki Halep ve İdleb' den çıkarılan uçucu yağlar benzer antimikrobiyal etkiye sahip olduklar inden benzer bikşıklere sahip oldukları düşünlm ektedirr.

Anahtar Kelimeler: Aromatik bitkiler, uçucu yağlar, MIC, MBC, MTC.

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CHAPTER 1

INTRODUCTION

Aromatic plants and essential oils are used for the treatment of diseases and food preservative and are used in flavoring. Positive effects on health; there is an increase in the use of synthetic additives in developing technology in the food industry, there are different negative effects of these synthetic additives on public health and the consumers escape from the consumption of the food containing these has facilitated at natural antimicrobial in food production have use in animal feed and organic farming practices. Plant essential oils have activities of antibacterial, antimutagenic, antioxidative, antifungal and antiviral, and these activities are different according to the countries and geographical regions. Essential oils need knowledge about how to use the antimicrobial mortality rate instead of synthetic food additives, in the food protection antimicrobial disinfection is important. The essential oil of the thyme, mint, chamomile, anise, and basil that used in this study in dry form obtained from Turkey (Gaziantep) and Syria (Aleppo and Idlib) were obtained by Clevenger device using the method of hydro-distillation whose time is three hours then treat these essential oils against twenty species of microorganisms (six ones are Gram-negative bacteria, six ones are Gram-positive, six ones are molds and two ones are yeasts) to specify MIC, MBC, and MTC (Minimum Inhibitory Concentration, Minimum Bactericidal Concentration and Maximum Tolerance Concentration respectively). The objectives of this research; to determine the antimicrobial properties (MIC, MBC, and MTC) of the essential oils that obtained from Turkish and Syrian aromatic plants, to compare the effect of the Turkish and Syrian essential oil against the studied microorganisms, to show the essential oil's importance properties in food preservation, medicine, and health and to bring the attention of the role of the essential oil against the studied microorganisms that causes disease and food spoilage.

The antimicrobial values (MIC, MBC, and MTC) of the essential oil obtained from Turkish and Syrian aromatic plants compared and provided information about the impact of the essential oils against the studied microorganisms. The concentration used as antimicrobial agents in a system, although there are a lot of factors that affected the essential oil antimicrobial properties. Therefore, statistical analysis was done on the MIC, MBC and MTC data obtained in this research and essential oils having significantly different effects on microorganisms aimed to be determined. Effect of essential oil plant type, region obtained (Syria and Turkey), microbial classification on MIC, MBC, and MTC concentrations are analyzed through the One-Way ANOVA and independent sample t-tests at $\alpha=0.05$ level being $p<0.05$ significantly different from rest of data.

CHAPTER2

LITERATURE REVIEW

2.1. Aromatic plants

Aromatic plants are special type of plants that are used for medicinal purposes through their aroma and flavor. They have great economic value. Their components exist in wood, root, foliage, bark, fruit, seeds, flower, and seed. Examples of aromatic plants include anise, thyme, basil, chamomile, and mint.

2.1.1.*Pimpinella anisum (anise)*

It is an aromatic plant called *Pimpinella anisum* belong to the Apiaceaefamily and its origin is Southwest Asia and Eastern Mediterranean region. It could grow to more than 1 m in height. Its leaves are shallowly lobed, from one to five cm long and divide into many small leaflets. Its flowers are white, usually called "aniseed". Western cuisine uses anise to flavor dishes, candies and drinks. The highest powerful element of anise essential oil is anethole. In 1999, the global production of anise essential oil reached approximately 8 tons. According to the farming process the anise composition differs and the main components' percentage are fatty oil; 8-23%, protein; 18%, moisture; 9-13% , starch: 5%, and essential oil; 2-7% ,while the N-free extract is 22-28% and crude fiber is 12-25%. By distillation method, around 2-3% of essential oil produced and 80-90% of this essential oil is anethole.

2.1.2. *Ocimum basilicum (Basil)*

It is an aromatic plant called *Ocimum basilicum*, its family is *Lamiaceae*, its origin is probably India and for more than 5,000 years has been planted. Italian cuisine used basil

as a culinary herb prominently, in addition to Southeast Asian cuisine; such as Taiwan, Vietnam, Laos, Thailand, Cambodia, Malaysia, and Indonesia. Basil leaves may sometimes taste similar to anise depending on the type of soil and agricultural input used. There are many types of basil and others related to species and sub-species that called basil as a common name. There are various scents belong to the many types of basil because it has much different essential oil that differs in its ratio according to the basic types. The eugenol is the reason for the strong scent that related to the sweet basil and there is similar chemical to the real cloves. The scents related to the lime basil, lemon basil; as in lemon mint and limonene; expresse of their higher ratio of citrus and causes this impact in a lot of plants. And there is a strong camphor odor in the African blue basil because of the huge amount of camphene and camphor.

Licorice basil that called “anise basil” because it has the same anethole that is in anise and makes the basil and anise in the same odor.

2.1.3. *Matricaria chamomilla (chamomile)*

It is an aromatic plant belongs to the *Asteraceae* family that is used for many medicinal purposes by making herb fermentations in addition to its preparations’ using in hemorrhoid, ulcers, hay fever, gastrointestinal disorder, insomnia, menstrual disorders, muscle spasm, and inflammation. The cultivated lands of Chamaemelum nobile are in North America, Argentina, and in Europe. Nobile is, along with Matricaria chamomilla, is a significant source of the herbal produce identified as chamomile. Nobile has daisy-like white flowers that appear during summer and has a herbaceous, fruity, crisp and sweet fragrance. It is used to perfume cosmetics, flavor foods, and in herbal teas. In order to create a fragrant chamomile lawn, seeds need to be distributed at an average of 83-100 per square meter. The lawn is only appropriate to light foot traffic or in places where mower access is hard.

2.1.4. *Thymbra spicata (thyme)*

It is an evergreen herb that has several usages in cooking, ornament, and medicine. It presents in the family Lamiaceae and *Thymus vulgaris* is the most common variety. Suitable conditions for thyme cultivation are hot location, sunny weather, well-

drained soil. The dividing of roots or cutting of seeds can make thyme spread. Thyme endures the bad weather conditions grows on mountains uplands. Thyme is considered as a vital component in the condiment zahtartar; Arabic for thyme, especially in some Levantine countries. Two forms of thyme; fresh and dried, the fresh one exists year round and can be soled in these two forms, although the fresh one can be stored for more than one week; it is tastier and can be frozen. Thyme may be measured by the spring, the bunch (or fraction thereof), teaspoon or tablespoon. The essential oil in the thyme reaches to 20-40% of *thymus vulgaris* and there are other components like linalool, borneol, and p-cymene. In addition to the thymol which is an active component in some all-natural alcohol-free land sanitizers and against many molds that cause contamination toenails.

2.1.5. *Folium menthae (mint)*

It is an aromatic plant called *Folium menthae* belongs to the *Lamiaceae* family. It has almost from 13 to 18 species and there is no significant difference between them. Part of these species have been hybridizing in natural methods and a lot of hybrids species are identified as a result. It has international distribution across Australia, Northen America, Asia, Africa, and Europe. The leaves have different colorsrelated to the green leaves themselves are in opposite paris; its flowers are white to purple, and its fruit consists of 1-4 seeds. The moist soils and wet environments are suitable for mint growing and grow in sun exposure all year round (Lillehoj et al., 2011). Mint can thrive close to rivers, pools, lakes, cool moist spots in partial shade; its tall reaches to 120 cm. The mints' growing is fast for this reason one plant can offer enough mint for home use. During the whole year, mint can be harvested then fresh leaves can be stored or used directly.

2.2. Microorganisms

2.2.1. DifferenceBetween Gram-Negative and Gram-Positive Bacteria

Gram-positive bacteria have a large peptidoglycan layer in the cell wall. Spores can be produced by Gram-positive bacteria under unfavorable environmental conditions. The spores increase exposure to bacteria to dangerous conditions and can cause foodborne diseases and pseudomembranous colitis. The outer cytoplasmic membrane

of the Gram-negative bacteria which lead to additional permeability barrier and outcomes of transport mechanisms across this membrane. The endotoxin is specific to cell Wall of Gram-negative bacteria and considers a major part of the cytoplasmic membrane and very necessary for the survival of bacterial and has the species-specific O antigen (also polysaccharide), the lipid A and core polysaccharide.

2.2.1.1. *Escherichia coli*

Gram-negative bacteria, its shape is a rod, facultatively anaerobic, prefer to live in warm-blooded organisms at the lower intestine and can be expelled to the environment through fecal matter. Although most of *E. coli* are not harmful, some of them lead to dangerous poisoning in the food. The harmless ones can produce vitamin K2 and prevent the pathogenic bacteria to make colonies and these strains are a normal part of the gut. Under aerobic conditions and during three days *E. coli* grows largely in fresh fecal matter, then the numbers decrease slowly later. E. *coli* and other facultative anaerobes cause the diseases through the fecal and oral transmission which is the main way for that and they form 1% of gut flora. Because *E. coli* cells have the ability to survive outside the body for little time; they become potential indicator organisms to determine the fecal contamination. The *E. coli*medium must have the energy and carbon like water, ammonium phosphate, magnesium sulfate, glucose, potassium phosphate, sodium chloride, monobasic, and dibasic. *E. coli* spend only twenty minutes to reproduce Under suitable conditions.

2.2.1.2. *Salmonella*Typhimurium

It is a Gram-negative bacteria rods, facultative anaerobic, motile, caused by people who are chronic holders and excretes (Typhoid) a status fecal-oral transmission can be contaminated by untreated cases in 1-3% of them.

2.2.1.3. *Yersinia enterocolitica*

It is Gram-negative bacteria, associated with polluted water and food especially meat and milk, on the host cell, the bacteria bind with attack proteins on their surface, this lets them be phagocytosed.

2.2.1.4. *Pseudomonas aeruginosa*

A common bacterium which leads to diseases in plants, animals, and humans. *P. aeruginosa* is rod-shaped. *P.aeruginosa* has significant medical importance, resistant to various drugs. This bacterium is very common and has mechanisms that resist antibiotics. This ubiquitous bacterium has been linked to the hospital-acquired infections such as a variety of sepsis syndromes and ventilator-associated pneumoniae. The bacterium takes advantages of a weakened body and capitalizes and dominates existing diseases to make matters much worse such as in the cases of cystic fibrosis and third and fourth-degree burns. It is also found generally in people with impaired immune systems but can infect people with normal immune systems, infections can be difficult because of its resistance to antibiotics. Its environments are soil, skin, water, droppings of the domestic cockroaches and exoskeletons, hospital settingsand the ones made by a human. In the normal and atmosphere with low oxygen can be thrived, thus has colonized many natural and artificial atmospheres. Generalized inflammation and sepsis are some of the symptoms and the results can be killed in case the colonization happened in the kidneys, urinary tract the and lungs (Sánchez E et al., 2010).

2.2.1.5. *Shigella dysenteriae*

It is Gram-negative rods, facultatively anaerobic, motile, not normal flora for the human body. It is transmitted by the fecal-oral route. It is an intracellular bacterium. It invades cells of gut lymphoid follicles, intestinal epithet cells on the basolateral surface, kill resident macrophages, then spread from cell to cell.

2.2.1.6. *Klebsiella pneumoniae*

Klebsiella pneumoniae is a Gram-negative that shape is rod nonmotile, lactose-fermenting, facultative anaerobic encapsulated, and on MacConkey agar it produces mucoid colony and ferment lactose. Although existance in the skin and mouth (Deans and Ritchie., 1987). During aspirating it by human or animals it can destroy the lungs. It becomes a significant pathogen in nosocomial infections last years. In anaerobic conditions, 30% of these strains can fix nitrogen(Hammer et al., 1999).

Naturally, two types of antigens on the cell surfaces can be expressed by some members of the *Klebsiella* genus naturally express two;the firstis *Klebsiella* antigen which is a capsular polysaccharide with more than 80 types. The second one is an element of the lipopolysaccharide (LPS) (Fraenkel., 1959). Both of them cause diseases and make the basis for serogroup.

2.2.1.7. *Listeria monocytogenes*

*Listeria monocytogenes*t is Gram-positive, non-encapsulated rod-shaped and "tumbling" motile bacterium, its growing related to several conditions, facultative intracellular parasite, can be transmitted through pasteurized milk/products improperly. It produces an extracellular product listeriolysin O and it is responsible for pathogenicity. Its cytolysin that exactly dissolves the endosomal membrane so that it avoids the essential anti-bacterial activity of the cell. Actin polymer allows bacteria to move in the cytoplasm and is necessary for cell-cell spread and makes it resistant against the humoral immunity.

2.2.1.8. *Staphylococcus aureus*

Staphylococcus aureus is Gram-positive bacteria, formed in clusters and non-molitle bacterium. It presents on human skin and nares, body walls, *S. aureus* causes pus formation to secrete 4 hemolysins that lyse cells and coagulases blood.

2.2.1.9. *Bacillus cereus*

*Bacillus cereus*is Gram-positive, beta hemolytic, non-encapsulated, motile, exists in soil and water, transmitted through the contaminated dishes of rice or meat and secretes enterotoxins.

2.2.1.10. *Bacillus subtilis*

Gram-positive bacteria, facultative aerobe and rod-shaped found in the gastrointestinal of humans and the ruminants. It used by biotechnology companies at

an industrial level and in addition to producing the enzyme. Although some of these bacterial strains can cause foodborne diseases with the production of polysaccharides in bread dough and producing ropiness. It can survive under the cooking conditions and still the best studied Gram-positive bacteria.

2.2.1.11. *Enterococcus faecalis*

It is a Gram-positive coccus, it has low pathogenicity and in the normal flora of the human gut. the resistance of the antibiotic is due to the conjugal transfer of antibiotic resistance genes and within species

2.2.1.12. *Streptococcus thermophilus*

It is a Gram-positive bacterium. In these bacteria the cell division happens along a single axis, so, the growth occurs in pairs, chains a lot of cases of necrotizing fasciitis happened because of this bacterium, (Tajkarimi et al., 2010) according to the hemolytic properties, species of *Streptococcus* are classified. (Boyle, 1955). The iron oxidization in hemoglobin molecules with red blood cells causes the greenish color of blood agar which is of the Alpha-hemolytic species. The rupture of red blood cells is caused by the Beta-hemolytic species. This will appear widely that is without the cells of blood around the bacterial colonies on blood agar.

2.3.Essential oils

Essential oils which extracted from aromatic plants by fragrance extraction methods like distillation, maceration, or pressing, are volatile aroma compounds, liquid and highly multipart mixtures of often hundreds of individual aroma composites. Its odor is the reason to be used in the perfumery and flavoring of the food.Essential oils have low solubility in water because they are not oils in a strict sense, the distinguishing between essential oils and aroma oils (in an oily solvent), absolutes, infusions in herbal oil and concretes.

2.3.1. Components of Essential Oils

The essential oils (EOs) extracted from aromatic plants' part like fruits, seeds, bark, flowers, plant roots, leaves, wood and peel (Hammer, 1999; Gann, 2013; Mith et al., 2014). They used from the past in the perfumes, medical purposes, and foods. It's organic components that do not participate directly in the development and normal growth or plant's reproduction and consider as secondary metabolites in the aromatic plants(Sartoratto et al., 2004). Sometimes these secondary metabolites participate in the defense of plants so, they may have antimicrobial properties(Sartoratto et al., 2004; Erkmen., 2003; Mathlouthi et al., 2012) found that when added the rosemary or oregano essential oil to broiler diets; they have the same impact for growth but in the laboratory against microorganisms' bacteria had a different antimicrobial impact. A combination of lemon, thymol, carvacrol, and eucalyptol was verified for their capability to avoid colonization and shedding in broilers intentionally fed *Salmonella enterica* sub sp. *enterica ser*. Heidelberg. Though, there was no significant decline, cecal colonization, and shedding (Cerisuelo et al., 2014). Broilers were fed an essential oils mixture consisted of thymol and cinna aldehyde, with or without butyric acid. They determined that the EO blend decreased cecal numbers of *Salmonella*, especially when mutual with butyric acid, as compared to control feed. An overview of the anti-Salmonella impact on the essential oils in agriculture was provided by Ricke et al., 2015. The essential oils' impact in laboratory rumen microbial fermentation were examined by Benchaar et al., 2007. They indicated that the phenolic composites (such as carvacrol, eugenol and thymol) affected ruminal fermentation, increased pH and decreased propionate, indicated antibacterial activity. (Callaway et al., 2008) studied the in vitro effects of orange peel and orange pulp essential oils against *Escherichia coli* O157: H7 and *Salmonella*Typhimurium in rumen fluid. The growing of both pathogens was decreased by the addition of 0.002 g/ml of orange peel or pulp. Callaway et al., 2011 noticed that the population of *S*. Typhimurium is reduced in the gut of the inoculated sheep experimentally when they feed on the products of orange peel with a significant reduction decreased in the ceca. Because of the customers' desire to reduce the using of the "unnatural or hazard chemicals" the antimicrobial properties of essential oils concentrate on the agriculture applications. (Schifferstein., 1998; Williams et al., 2001; Williams et al., 2004). In spite of a good number of essential oils researches and its antimicrobial

activities; just part of them were concentrating on the style action of these composites; so, the detailed knowledge of the antimicrobial properties are required with a style of action and antimicrobial method of action especially during application of essential oils as antimicrobial materials as food preservatives.

2.3.2. Impact of Essential Oils on Cell Membrane and Wall

There is a strong linkage between the essential oils and their hydrophobicity through their antimicrobial activities(Dorman et al., 2000; Ultee et al., 2002). The main component of the cell wall is peptidoglycan linked with other molecules such as proteins and teichoicin Gram-positive bacteria (Nazzaro et al., 2013). The hydrophilic lipopolysaccharides (LPS) are in the outer membrane of Gram-negative bacteria and create a wall against the hydrophobic composites like those that existed in essential oils (Nikaido et al., 1994 and 2003).The essential oils' impact against the Gram-positive bacteria are higher than the impact on the Gram-negative bacteria(Trombetta et al., 2005). The hydrophobic ingredients of the essential oils can reach to the periplasm of Gram-negative bacteria through the porin proteins of the outer membrane (Helander et al., 1998), through which they can slowly transportable (Plesiat et al., 1992).

2.3.3. Potassium Leak from Cells

The leak of potassium into the extracellular space is one of the most indicators for the growth in membrane permeability and the final loss of viability for the cell (Plesiat et al., 1992; Ultee et al., 1999; Cox et al., 2000; Lambert et al., 2001; Cox et al., 2001; Walsh et al., 2003; Fitzgerald et al., 2004; Inoue et al., 2004; Togashi et al., 2008; Bouhdid et al., 2010). The most important component of several essential oils that contains 60–74% of oregano and 45% of the them is carvacrol (Lagouri et al., 1993; Arrebola et al., 1994; Ultee et al., 1999) studied the antimicrobial properties of carvacrol against *Bacillus cereus* and determined that carvacrol caused a direct increase in the extracellular potassium and reduction in intracellular potassium at a concentration of 0.15 ml/l. (Fitzgerald et al., 2004) studied the effects of vanillin and carvacrol on *E. coli*, *Listeria innocua*, and *Lactobacillus plantarum*; they found that the extracellular potassium levels increased and intracellular potassium levels decreased in all organisms at concentrations 0.496 and 7.6 ml/l of vanillin and

carvacrol, respectively. The extracellular potassium at concentrations of 0.50 ml/l can be increased by thymol and eugenol in *S. aureus* and *E. coli*and 100% of the total cellular potassium in *E. coli* was released during half hour by tea tree oil at 2.50 ml/l, but just around 20% was released by *S. aureus* in the same time (Cox et al., 2000). (Bouhdid et al., 2009) found that the essential oil of oregano caused potassium leakage in both *S. aureus* and *P. aeruginosa*. Oil-treated cells were studied by transmission electron microscopy (TEM), which exposed mesosome-like coagulated cytoplasmic material, and structures intracellular material outside the cell; these effects were more visible in *P. aeruginosa* compared with *S. aureus* (Bouhdid et al., 2009 and 2010) found that a concentration of 1.25 ml/l was able to raise extracellular potassium levels in both bacteria and determine the effects of EO of *cinnamomum verum* on *P. aeruginosa* and *S. aureus* . (Inoue et al., 2004) tested the antimicrobial effects of terpene alcohols farnesol, plaunotol and nerolidol on *S. aureus*. When applied to S. aureus at a concentration of 0.020 ml/l, all these three components increased the extracellular potassium levels. (Togashi et al., 2008) studied the ability of farnesol to cause potassium leak by adding geraniol to farnesol in a ratio of 0.010:0.005 ml/l, and found that the potassium leak was improved, while the activities of leak of farnesol, geranyl inhibited. (Bajpai et al., 2015) used scanning electron micrographs of *B. cereus* and *E. coli* and the results were that the cells, which treated with *Ginkgo biloba* EO (250 and 500 µg/ml, respectively), had disruption of cell membranes and swelling of the cells which led to the leaking of potassium from the cells.

2.3.4. Leakage of Other Cell Components

People who are attempting to determine the microbial action of EOs are also interested in the release of other cellular components. Carboxyfluorescein diacetate is normally used in order to probe for viable cells, (Clerck et al., 1994; Veal et al, 2000). Carboxyfluorescein diacetate was used to stain live cells of *L. innocua* which were then showing to the EOs of *cymbopogon citratus*, *Thymus vulgaris*, or *Ocimum gratissimum* (Erkmen et al., 2015). The leakage of carboxyfluorescein from within the cell and thus a measure of the disturbance of the cell membrane was determined by the loss of fluorescence. EOs of *O. gratissimum* (5.0 ml/L), *T. vulgaris* (4.30 ml/L), and *C. citratus* (7.80 ml/L) were adjusted to a concentration of

1.04×10^4 arbitrary units (AUs) for treatment; AUs were determined by the formula AU ml^{-1} = mean diameter (mm) of the minimal inhibition zone × dilution factor × 50. All EOs were shown to cause a reduction in fluorescence as compared to an unexposed control (Nguefack et al., 2004); Xu et al., 2008) and they also studied the leakage of carboxyfluorescein by *E. coli* after exposure to components of thyme EO, thymol and carvacrol, at different concentrations (0.10 and 0.20 ml/l) and they found that both thymol and carvacrol caused an increase in carboxy fluorescein released by *E. coli* cells. Appraisal the release of carboxyfluorescein from large unilamellar vesicles (LUVs) that were added to linalyl acetate, (+) menthol, and thymol at different concentrations (0.10, 0.25, and 0.50 ml/l). They found that the release of carboxyfluorescein from the LUVs caused by thymol and (+) menthol, with less impact comparing with linalyl acetate (Trombetta et al., 2005). There were researches on the release of fatty acids from *E. coli* cells treated with carvacrol, thymol, (+)-carvone, or trans-cinnamaldehyde. It was determined that a significant amount of fatty acids was released into the supernatant by Carvacrol and thymol, while trans-cinnamaldehyde and (+)-carvone did not release fatty acids(Helander et al., 1998). (Helander et al., 1998) published the findings, which demonstrated that thymol and carvacrol break down the cell membrane. Proteins absorb light at 280 nm; therefore, the release of 280 nm materials measured was a positive indicator of macromolecular leakage by the cell; *E. coli* and *S. aureus* exposed to tea tree oil were found to 280 nm absorbing the material, but at levels lower than potassium leakage (Cox et al., 2001). On the other hand, nucleic acids absorb light at 260 nm. The release of 260 nm material from bacterial cells would be an indicator of the macromolecular leak by the cell (Ifesan et al., 2009; Carson et al., 2002; Oussalah et al., 2002).Each one these people studied the release of 260 nm absorbing material. Strains of *S. aureus* exposed to a crude Eleutherine Americana had a peak release of 260 nm absorbing materials after eight hours of exposure to 2.50–10.0 ml/l of the extract (Xu et al., 2008). After 22 hours of exposure, the absorption rate changes noticeably. Due to denaturation of the materials, which caused them to become unreactive to the light at 260 nm, the absorbance reduced from the levels seen after eight hours (Xu et al., 2008). A significant amount of 260 nm material was released by *S. aureus* cells after an hour of exposure to tea tree oil or its components (Ifesan et al., 2009). (Oussalah et al., 2006) examined savory, and Spanish oregano and Chinese cinnamon EOs against *E. coli* O157: H7 and *L. monocytogenes* and they

found that at 0.25 ml/l each EO was able to cause an increase in the release of 260 nm absorbing material. The effects of the EOs of oregano and rosemary on *Pseudomonas fluorescens* include cell material released into the growth medium immediately after contact with either EO separately or in combination (Oussalah et al., 2007).

2.3.5. Uptake of Substances

The cell membrane assistances cells to have an important role in controlling what enters the cell and what exists out of it. When ions are not being regulated, they will have formed in the cell membrane at the very least small pores. When larger molecules, like propidium iodide (PI) or N-phenyl-l-naphthylamine (NPN), are inserted without regulation inside the cell, it's an indicator to increase the possibility of cell death and larger pores in the membrane will be formed. (Helander et al., 1998) studied the absorption of NPN by *E. coli* and *S*. Typhimurium after exposure to thymol, trans-cinnamaldehyde, carvacrol, and (+) -carvone. The uptake of NPN by both by *E. coli* and *S*. Typhimurium significantly increased by thymol and Carvacrol, but not with trans-cinnamaldehyde and (+)-carvone. That shows that the trans-cinnamaldehyde and (+)-carvone cannot form pores to be enough for NPN, contrary thymol and carvacrol have a similar minimal inhibitory concentration (MIC), according to Helander's study, while Trans-cinnamaldehyde does not have the impact as a large pore former like carvacrol and thymol. This guide to the fact that there must be another mode of action for trans-cinnamaldehyde. (Fisher and Phillips, 2009) also studied the role of *E. faecium* and *E. faecalis* by up taking the NPN after adding to the oil blend of citrus with lead to increase the NPN uptake over twofold, by oil blend so, the oil blend causes great pore formation in the bacteria. Some changes in the cell happened after contacting with essential oil or its vapor. The cells that contacted with essential oils have vacuoles, which contained the EO within them, according to the authors' speculations. After mixing with oil that extracted from the tea tree, almost 10% of *S. aureus* cells and 100% of *E. coli* cells showed increased PI uptake (Cox et al., 2001). Cox's results show differences in the impact of tea tree oil on Gram-positive bacteria and Gram-negative bacteria (Cox et al., 2001). Similarly, (Bouhdid et al., 2010) identified that the essential cinnamon's oils have more impact against *P. aeruginosa* than *S. aureus* related to PI uptake.

2.3.6. Effects on Membrane Potential

The cell used the membrane potential to get the lifelike synthesis of nucleic acids, polysaccharides, enzymes, and other components of the cell to repairing cell repair and maintenance of damage (Fisher et al., 2009). If the membrane potential decreases that means damage in the cell membrane. (Bouhdid et al., 2010) used bis-oxonol dye to stain depolarized *S. aureus* and *P. aeruginosa* cells. They found that the essential oils that extracted from the cinnamon can reduce the membrane potential of *P. aeruginosa*, but not *S. aureus* at MIC levels was by using 3,3′-dipropylthiacarbocyanide iodide (DiSC35) to control membrane potential. (Veldhuizen et al., 2006) Found that by adding the essential oil's components like o-cresol, carvacrol, or 2-amino-p-cymene against *S. aureus* at MIC concentrations, the membrane potential of the cells was reduced.

2.3.7. Effects on ATP: Leakage from Cells and Usage by Cells

(Ultee et al., 2002) determined that cymene, a plant aromatic compound found in cumin, cannot cause ATP levels to decrease in the intracellular environment or increase in the extracellular environment. Although, during twenty minutes of contact the carvacrol delete the internal ATP of *B. cereus* cells at 0.150 ml/l concentration. (Fisher and Phillips, 2009) used the mixture of essential oil that contains a 1:1 mixture of bergamot and sweet orange to deal *E. faecium* and *E. faecalis* and found that, during ten minutes of contact, the internal ATP was dissolved by the mixture. (Oussalah et al., 2006) studied the impact of Spanish oregano, Chinese cinnamon, and savory EO on the ATP of *E. coli* O157: H7 and *L. monocytogenes* and found an increase in the levels of the extracellular by Savory EO was in both bacteria, and by Spanish oregano in *E. coli* O157: H7, while there was no increase in the extracellular ATP when Chinese cinnamon was used in either organism. (Helander et al.,1998) studied at the impact of essential components like carvacrol, thymol, (+)-carvone, and trans-cinnamaldehyde on the ATP of *E. coli* cell and *S.* Typhimurium and found that, carvacrol reduce intracellular ATP more than 20-minutes period and increase external ATP, but there is no impact on the ATP of the cells, externally or internally by (+)-carvone and trans-cinnamaldehyde . (Gill and Holley, 2004) examined ATP generation in *L. monocytogenes* and *L. sakei* that

treated with eugenol, and *L. monocytogenes* was also treated with cinnamaldehyde, eugenol was found to inhibit ATP production but was unable to reduce intracellular ATP, cinnamaldehyde was found to both inhibit production of ATP and reduce intracellular ATP so, these results were determined by mixed the essential oils to the bacteria before or after the addition of 0.25% glucose for cell's motivations,they propose that the mode of action for eugenol depend on inhibiting the cell from using glucose because of its effectiveness when added prior to or after glucose, cinnamaldehyde reduced ATP levels when added either prior to or after glucose, which led Gill and Holley to suppose that there may be more than one reasonable mode of action. Carvacrol, cinnamaldehyde, and eugenol have been found to inhibit the membrane-bound ATP as bacteria's activity. There are many enzymes with ATPase activity that are related to bacterial membranes, including one that is involved in ATP generation and cellular pH regulation (Gill and Holley, 2004).Gill and Holley supposed that ATP inhibition plays an important role in reducing the growth rate of pathogens at sublethal concentrations of the essential oils.

2.3.8. Effects on pH Gradient

It is necessary maintaining a pH gradient for the survival of cell and it is also an indicator to know the percentage of damages in the cell and there are some studies about the impact of the essential oils on the the internal pH of bacteria and found that Chinese cinnamon, Spanish oregano, and Savory EOs can reduce the internal pH of *E. coli*O157: H7 and *L. monocytogenes* (Lambert et al., 2001; Oussalah, 2006). (Fisher and Phillips, 2009) used 1:1 mixture of essential oils of orange and bergamot (20.0 ml/l of each) to determine that the mixture remove the pH gradients of *E. faecium* and *E. faecalis* and. (Fitzgerald et al., 2004) studied *L. plantarum* with vanillin and determined that the pH gradient was dissipated at 15.2 ml/l of vanillin. A lot of essential oils have the impact that makes the cell unable to maintain a pH gradient that is very necessary for the generation of a proton life and its motive force.

2.3.9. Effect on Respiration

The most important part of aerobic metabolism is the respiration and it is a potential target for antimicrobial of essential oils, so a lot of studies concentrated on the

impact of essential oils against the respiration. One indicator of the activity of the respiratory enzyme is the reduction of 5-cyano-2,3-ditolyl tetrazolium chloride (CTC).(Bouhdid et al., 2010) determined that after a 30-min exposure to the MIC of the oil in the existence of cinnamon essential oil less than 6% of *P. aeruginosa* cells decreased CTC at the concentration of (1.25 ml/l). However, *S. aureus* was found to be more resilient as about 10% of the cells were able to decrease CTC after 60 min of exposure to the MIC (1.25 ml/l) and 1.5× MIC (1.87 ml/l) of the oil. (Cox et al., 2000) studied the oxygen consumption of *E. coli* and *S. aureus* in the existence of tea tree oil at different concentrations; 5.0 ml/l of tea tree oil reduced the oxygen consumption of *E. coli* by 100% and 10.0 ml/l reduced the oxygen consumption of *S. aureus* by 60%. (Fitzgerald et al., 2004) studied the consumption of oxygen in *L. innocua* and *E. coli* and found that oxygen consumption was reduced by 52% and 19% in *L. innocua* and *E. coli* respectively, when exposed to 6.09 ml/l of vanillin. So, some of the essential oils disrupt the ability of bacterial cells to perform oxidation-reduction reactions.

2.3.9. Effect on Quorum Sensing

Quorum Sensing (QS) is cell-to-cell communication among bacteria. It is accomplished using small signaling molecules produced by the bacteria. Bacteria utilize (QS) to evaluate their internal physiological status and external environment (Gill et al., 2006).

Autoinducers are generally the signaling molecules Gram-positive bacteria use modified oligopeptides as signaling molecules; whereas the Gram-negative bacteria use acyl homoserine lactones, QS is involved in swarming, stress resistance, biofilm production, virulence, and motility (Miller et al., 2001). Researchers work for using QS as antimicrobial resistance and, also, as a way to decrease the spoilage of food (Kjelleberg et al., 2002). QS is also thought to let pathogens to weaken the host immune responses by postponing the production of virulence factors until the bacterial population be huge enough to overwhelm host protection mechanisms and create infection (March et al., 2004). Khan et al., 2009) used Chromo bacterium violaceum CV12472, CVO26 and biosensor strains to screen 21 EOs for anti-QS activity. The results concluded that the most anti-QS activity on both mutant and wild strains was established by clove oil, followed in activity by lavender,

cinnamon, and mint oils. The effect QS controlled- clove oil on pigment production, was found that it is dependent on concentration levels. At sub-MICs of clove oil, there was a 78.4% decrease of pigment production compared with the control and up to a 78% decrease in swarming motility over the control (Khan et al., 2009). (Szabo et al., 2010) achieved similar studies with a wider range of EOs and established that rose, lavender, rosemary and geranium oils were the most potent QS inhibitors of the EOs they tested. Eucalyptus and citrus oils moderately reduced pigment production by CV026, while juniper, orange, and chamomile, oils were ineffective. (Alvarez et al., 2014) studied the use of oregano EO in eatable films and the effect on QS as well as the antibacterial activity against many pathogens. Pectin films incorporated with 25.90 and 36.10 ml/l of oregano EO inhibited the growth of *E. coli*O157: H7, *L. monocytogenes*, *Salmonella cholerasuis*, and *S. aureus*. All concentrations of oregano EO (0.015, 0.0312, 0.0625, and 0.125 ml/l) and pectin-EO films (15.70, 25.90, and 36.10 ml/l) showed an important anti-QS activity. They also determined that the pectin-EO films reduced total coliforms, yeast, and molds on shrimp and cucumber slices stored for 15 days at 4°C. Thus, EOs could be used to explicitly target QS in a novel antimicrobial strategy.

2.3.10. Effect of Composite Structure on Antimicrobial Activity

Several researchers have studied the structures of EOs themselves to determine what allows these composites to affect the many cellular activities of bacteria. (Sikkema et al., 1994) studied how biological membranes respond to cyclic hydrocarbons and determined that the hydrocarbons accumulate in the inner of the membrane causing it to swell. The amount to which a particular hydrocarbon can accumulate in the membrane was found to be directly linked to its membrane partition coefficient, they proposed that the accumulation of these compounds in the membrane result in changes to the membrane function and structure, thus causing inhibition or cell death. Carvacrol is an EO that has been shown to have high antibacterial activity and has been the focus or benchmark for several studies, some of which have studied what particular property of the carvacrol structure makes it antimicrobial. Carvacrol is a cyclic hydrocarbon which, according to (Sikkema et al., 1994) allows it to accumulate in the membrane. It also possesses a hydroxyl group on the ring, which may be responsible for the antimicrobial activity of carvacrol (Ultee et al., 1994). (Ultee et al., 2002) also studied menthol, cymene, thymol carvacrol methyl ether, in

addition to carvacrol, these composites have similar structures to carvacrol that only differ in one or two way,*Salmonella*Typhimurium has the same chemical formula as carvacrol, but the hydroxyl group is in the meta position rather than the ortho position,menthol lacks the benzene ring and instead has a cyclic hexane ring, carvacrol methyl ether has the hydroxyl group replaced with a methyl ether group so, finally, cymene completely lacks a hydroxyl group and they noted that cymene and carvacrol methyl ether lacked any antibacterial activity, menthol showed slight inhibitory activity at a concentration of 0.320 ml/l for up to 5 h, but after 24 h, the bacteria had rebounded to levels seen with only a 0.016 ml/l concentration of menthol, which showed no inhibition of the bacteria, thymol showed a very similar pattern of activity to that of carvacrol, with concentrations above 0.113 ml/l completely inhibitory to the bacteria. (Veldhuizen et al., 2006) achieved similar results when they examined o-cresol, 3-isopropyl phenol, 2-amino-p-cymene, p-cymene, and 3,4-dimethylamine in comparison to carvacrol. They found that p-cymene and 3,4-methylcumene exhibited no antibacterial activity. In 3,4-methylcumene, there is a methyl group in the place occupied by a hydroxyl group in carvacrol, indicating that the hydroxyl group is an important component of the antibacterial activity of carvacrol. The compound 3-isopropylphenol, which lacks the methyl group on the ring, was found to have a MIC approximately 1.5 times higher than that of carvacrol, while o-cresol, which lacks the isopropyl group of carvacrol, was found to have a MIC approximately two times higher than that of carvacrol. The MIC of these two carvacrol-related compounds indicates that the R groups do play some role in the antibacterial activity of carvacrol, though not as much as the hydroxyl group. The compound 2-amino-p-cymene has an amino group in the same position as the hydroxyl group of carvacrol, but the rest of the structure is similar. The MICs of 2-amino-p-cymene were approximately 0.450–0.60 ml/l higher than that of carvacrol and had the highest MIC of all antibacterial active compounds tested. Since 2-amino-p-cymene shows antibacterial activity, it seems that the hydroxyl group is not essential for antimicrobial activity, although it appears to enhance the antimicrobial activity considerably. (Ben Arfa et al., 2006) also examined how the structure of carvacrol affects its antibacterial activity. They compared carvacrol with carvacrol methyl ether, carvacrol acetate, eugenol, and menthol. Carvacrol methyl ether and carvacrol acetate were not found to display any antibacterial activity; both of these compounds have substitutions at the hydroxyl

group of carvacrol respective of their names, indicating that these groups cannot convey the same activity as the hydroxyl group of carvacrol. Eugenol, which is another aromatic compound like carvacrol, has a hydroxyl group and an ether group which is in a para and ortho position, respectively, to a three-carbon chain that ends in a double bond. Eugenol was found to exhibit lower antibacterial activity than carvacrol but was not inactive like carvacrol acetate and carvacrol methyl ether. (Ben Arfa et al., 2006) only determined MICs for their compounds, so it is difficult to say if the antibacterial action of eugenol is the same as that of carvacrol, although it can be said that not all antibacterial EO compounds must have a structure similar to that of carvacrol in order to possess antibacterial activity. Similarly other authors (Ultee et al., 2002; Ben Arfa et al., 2006) found menthol to possess very little antibacterial activity, which supports the theory that the benzene ring is important for antibacterial activity, but only for those compounds that possess a hydroxyl group or an amino group, as was the case with 2-amino-p-cymene in the study by (Veldhuizen et al., 2006).

2.4. Uses of Essential Oil in Food Preservation

The essential oils extracted from the aromatic plants are known as natural additives involving the antimicrobial and antioxidant agents. Their effects depend on the plant source, extraction methods, and chemical components. Using the essential oils in prepared foods still limited due to the specific smell related to the volatiles, which may modify the foods' flavor.

2.4.1. Antimicrobial Activity

Protect foods against spoilage and pathogenic microorganisms by the essential oils has been highlighted (Lis-Balchin et al., 1998; Friedman, 2006; Rojas-Graü et al., 2007). Carvacrol, one of the chemical elements in numerous essential oils, has been exposed to apply as diverse antimicrobial factor (Veldhuizen et al., 2006). It is the main compound of the essential oil from oregano (60% to 74%) and thyme (45%) (Lagouri et al., 1993; Arrebola et al., 1994). In addition, (Friedman et al., 2002). Carvacrol has an effect of antimicrobial activity against most gram-negative and gram-positive bacteria; the breaking of the outer membrane of gram-negative bacteria by Carvacrol leads to release lipopolysaccharides and increase the permeability of the cytoplasmic membrane to ATP.(Burt, 2004). (Veldhuizen et al,

2006) demonstrated that the Gram-positive bacteria can react with the bacteria's membrane and change the permeability for cations H+ and K+. Generally, the main antimicrobial activity of the essential oils is related to gram-positive more than to gram-negative bacteria (Kokoska et al., 2002; Okoh et al., 2010). Lipophilic ends of lipoteichoic acids in cell membrane of gram-positive bacteria may enable the permeation of hydrophobic compounds of essential oils (Cox et al., 2000). The confrontation between the gram-negative bacteria andessential oils is related to thecell wall lipopolysaccharides or extrinsic membrane proteins, that restricts the rate of hydrophobic composites movement through the lipopolysaccharide layer (Burt et al, 2004). (Ultee et al., 1999) reported that the dissipation of ion gradients leads to kill the cell through weakening the processes of essential oil in the cell. There are two principal functions to the cytoplasmic membrane of bacteria: First is the function barrier and the transduction of energy, allowing the membrane to form ion gradients, that use to drive many processes; Second is the formation of a matrix for membrane-embedded proteins, like the membrane-integrated complex of ATP(Sikkema et al., 1995; Hensel et al., 1996). The activities of the essential oil are associated with the functional groups, synergistic contacts between components and composition (Dorman and Deans, 2000). Exclusion of the aliphatic ring substituent of carvacrol leads to reduce the antimicrobial activities slightly. The higher antimicrobial activity of carvacrol, compared to other plant phenolics, could be explained by the fact that hydroxyl group in the structure of phenolic composites gives antimicrobial activity and its relative position is very critical for the effectiveness of these natural components (Veldhuizen et al., 2006). Rosemary (*R. officinalis*) essential oil exhibits both Gram-negative bacteria (*E. coli* and *K. pneumoniae*) and Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) (Okoh et al., 2010). The main elements of rosemary oil are monoterpenes like borneol, myrcene 1,8-cineole, α-pinene, β-pinene, verbinone, and camphor (Santoyo et al., 2005; Okoh et al., 2010), Which have strong antimicrobial activity through broking down of bacteria membrane integrity (Knobloch et al., 1989; Aguirre et al., 2013). (Burt 2004) and (Pelissari et al., 2009) also reported that essential oil that extracted from the oregano had advanced antimicrobial activity against the Gram-positive bacteria (*S. aureus*) than Gram-negative (*E. coli* and *P. aeruginosa*). γ-therpinene thymol, carvacrol, and ρ-cymene are the main components of oregano essential oil (Lambert et al., 2001; Burt, 2004; Aguirre, 2013). However,

Pseudomonas putidawas show resistant against the essential oils of parsley and carrot seed (Teixeira et al., 2013). *Salmonella* Typhimurium and *E. coli* were also tolerant of the essential oils that extracted from the grapefruit, carrot seed, parsley, lemon, and onion. The complexed double-layer cell membrane of Gram-negative bacteria against essential oils could be the reason for its resistance, Comparing with the single-layer membrane of Gram-positive bacteria(Hogg, 2005). Antimicrobial activity of the essential oil of Callistemon comboynensis was detected on Gram-positive bacteria (*B. subtilis* and *S. aureus*), Gram-negative (*Proteus vulgaris* and *P. aeruginosa*), and a pathogenic fungus Candida albicans. According to (Abdelhady and Aly., 2012) the high content of oxygenated constituents is the reason of this antimicrobial activity. They reported that the essential oil of *C. comboynensis* leave consists of 1,8-cineole (53.03%), eugenol (12.1%), methyl eugenol (8.3%), α-terpineol (4.3%) and carveol (3.4%), and. Furthermore, the maximum decrease (8.0 log cfu/ml) was gotten when the essential oils of rosemary, organum, and coriander were used at a level of 20 µL to inhibit *Listeria innocua* (Teixeira et al., 2013). Thyme essential oil at a level of 20 µL was able to inhibit both *L. monocytogenes* and *L.innocua*. Though, rosemary essential oil displayed the highest MIC (90.8 µg/mL) against *S.* Typhimurium Brochothrix thermosphacta. So, the essential oils that extracted from the aromatic plants can be used as antimicrobial agents for food applications and other purposes, and its antimicrobial activities depend on kinds of the used essential oil.

2.4.2. Antioxidant Activity

The plant phenols that are part of the essential oil's structure that has antioxidant activities and from all the essential oil components; the thymol and carvacrol have the highest antioxidant activities., (Dapkevicius et al., 1998). Essential oils can play its role as an antioxidant through prevention of the chain initiation, peroxides' termination, prevention of continuous hydrogen's abstraction, reducing agents, and quenchers of singlet oxygen formation and binding of transition metal ion catalysts (Yildirim et al., 2000; Mao et al., 2006). essential oils can play the role of potential natural antioxidants which stop the lipid oxidation in the system of food nutrition. The organic components such as phenolics that consist of a carbon atom that linked with a hydroxyl group (-OH) that can provide free radicals leading to oxidization of

the other compounds (Nguyen et al., 2003). The phenolic components have the capability of antioxidant because of the redox properties; which make it reduce the agents and singlet oxygen quenchers. (Kumar et al., 2005). The antioxidant activity is mostly related with the key activities of the essential oil compounds like m-thymol in thyme, eugenol in clove, carvacrol in origanum and β-citronellol or β-citronellal in citronella(Wei and Shibamoto, 2010; Bozin et al., 2006; Ruberto and Baratta, 2000; Bounatirou et al., 2007). in addition to the methyl chavicol, eucalyptol, (−)-bornylacetate, (−)-camphor, terpinene, and other components in the essential oils display antioxidant activities. (Ruberto and Baratta, 2000; Mitić-Ćulafić et al., 2009; Teixeira et al., 2013). The difference in the components of the essential oils are the main reasons for the difference in their antioxidative activities (Burt, 2004; Kordali et al., 2005). The extraction method of the essential oils and the used solvents have an impact on antioxidative activities. (Sarikurkcu et al.,2010) reported that free radical scavenging activity (DPPH assay) and dropping the power of essential oil from *Thymus longicaulis* subsp. Longicaulis var. longicaulis extracted via HD method was lower than those extracted using water or methanol. The methanol extract of Salvia tomentosa displayed higher radical scavenging activity to other extracts (IC50 = 18.7l μg/mL) (Tepe et al., 2005). The polarities of the phytochemicals have a key role in the antioxidative activities, for this reason, the polar extracts showed higher effective activities than nonpolar extracts. (Giweli et al., 2012) studied the antimicrobial and antioxidant activities of essential oils of *Satureja thymbre* growing wild in Libya were studied by (Giweli et al., 2012) and the results of antimicrobial and antifungal are given the Tables 2.1. and 2.2.

Table 2.1. Antibacterial activity of *Satureja thymbra*essential oil and their main compounds tested by microdilution method (MIC and MMC in µg/mL).

Bacteria	*Satureja thymbra*		***streptomycin***		***thymol***		***γ-terpenene***		***carvacrol***	
	MIC*	MMC*	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC
Micrococcus flavus	0.001	0.002	0.0005	0.001	0.025	005	0.05	0.07	0.0025	0.005
Bacillus cereus	0.05	0.1	0.0005	0.0005	0.025	0.05	0.05	0.07	0.0125	0.025
Pseudomonas aeruginosa	0.05	0.1	0.001	0.002	0.1	0.15	0.15	0.3	0.05	0.1
Listeria monocytogenes	0.1	0.2	0.001	0.002	0.1	0.1	0.1	0.2	0.05	0.05
Staphylococcus aureus	0.05	0.1	0.001	0.001	0.025	0.05	0.05	0.1	0.025	0.05
Escherichia coli	0.05	0.01	0.0005	0.001	0.1	0.15	0.15	0.2	0.05	0.05
Proteus mirabilis	0.05	0.1	0.001	0.002	0.01	0.15	0.15	0.3	0.05	0.1
***Salmonella* Typhimurium**	0.05	0.1	0.001	0.001	0.05	0.1	0.1	0.2	0.05	0.05

MIC: minimum inhibitory concentration, MMC: minimum microbicidal concentration

Table 2.2. Antifungal activity of Satureja thymbraessential oil and their main compounds tested by microdilution method (MIC and MFC in µg/mL)

Fungi	*Satureja thymbra*		***streptomycin***		***thymol***		***γ-terpenene***		***carvacrol***	
	MIC*	***MFC****	***MIC***	***MFC***	***MIC***	***MFC***	***MIC***	***MFC***	***MIC***	***MFC***
Aspergillus niger	0.025	0.05	1.5	2	0.01	0.02	0.02	0.03	0.025	0.025
Penicillium funiculosum	*0.025*	*0.05*	*2*	*2.5*	*0.0125*	*0.025*	*0.025*	*0.07*	*0.0125*	*0.0125*
Aspergillus fumigatus	0.025	0.05	1.5	2	0.025	0.05	0.05	0.07	0.025	0.025
Aspergillus ochraceus	0.025	0.05	1.5	2	0.01	0.015	0.015	0.02	0.005	0.01
Aspergillus flavus	0.025	0.05	1.5	2	0.01	0.01	0.02	0.03	0.005	0.01
Penicillium ochrochloron	0.001	0.001	1.5	2	0.025	0.025	0.025	0.07	0.0025	0.005
Trichoderma viride	0.025	0.05	2	2.5	0.01	0.01	0.05	0.07	0.005	0.01

MIC: minimum inhibitory concentration, MFC: minimum fungal concentration

2.5.Essential Oils' Extraction

The essential oils extracted from aromatic plants usually done by many methods of extractions like distillation, solvent and combination methods, these methods are being determined according to the used plant material and its form and state. In addition to that, the extraction method of essential oil is the most important indicators that may affect the essential oil's quality so, using the wrong method leads to altering and damaging the chemical signature's action of essential oil and losing in the natural bioactivity and characteristics such as discoloration, off-odor, and increased viscosity and other physical changes. Thus, all these unlikely changes must be avoided during the extraction of essential oils.

2.5.1. Distillation

2.5.1.1. Steam Distillation

Steam distillation method is one of the most well-known methods that use to extract the essential oil extracting from the plants (Reverchon and Senatore, 1992). 93% of the extracted essential oils are done by this method (Masango, 2005). Principally, applying this method, plant sample is added in boiling water to be heated by steam. then the structure cells of plant's material are broken by the heat that leads to releasing the compounds of aromatic plants (essential oils) (Perineau et al, 1992; Babu and Kaul, 2005). (Yildirim et al., 2004) reported that the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of essential oils from the steam distillation process were displayed greater than essential oils extracted by hydro distillation (HD) method.

2.5.1.2. Hydro Distillation

Hydro Distillation (HD) is well-known as the standard method to extract the essential oil extraction from part of plants like wood, flower, and leaves. The process of hydro distillation includes the total plant materials in water, boiling. HD keeps the extracted the essential oils to a sure degree as long as the nearby water plays as a wall to avoid it from overheating. The essential oil and steam vapor are condensed to an aqueous portion. One of the HD benefits is that the essential material could be distilled

under100°C. (Okoh et al., 2010) studied the outcome of several extraction methods on the properties of essential oil and yield from rosemary (Rosmarinus of- ficinalis L.) using Solvent-Free Microwave Extraction (SFME) and HD. They found that the total yields were 0.39% and 0.31% through SFME and HD, respectively. HD extracted oil (32.95%) monoterpene hydrocarbons and (26.98%) oxygenated monoterpenes, while SFME-extracted oil monoterpene hydrocarbons (25.77%) and oxygenated monoterpenes (28.6%). There are two advanced HD methods, Microwave-Assisted HD (MAHD) (Golmakani and Rezaei, 2008) and Ohmic-Assisted HD (OAHD) (Gavahian et al., 2012). MAHD utilize a microwave oven in the extraction process and save energy and extraction time 75 minutes, while HD needs four hours. OAHD method had an extraction time of 24.75 minutes, although HD spent one hour to extract the essential oil from thyme. There are no deviations in essential oils' components gotten by OAHD in comparing with HD.

2.5.1.3. Hydro Diffusion

The hydro diffusion extraction method is different from the steam distillation just in the inlet way of steam into the container of still. It is used for dried and not damaged plant material during boiling temperature (Vian et al., 2008). The application is done from the plant materials top while the steam is going from the bottom for this method. It could be used under low vacuum or pressure and the temperature less than 100°C. Hydro diffusion method is superior to steam distillation because of the less time of extraction, higher oil yield and less used steam. (Bousbia et al., 2009) Comparing between HD and MHG (Microwave Hydro diffusion and Gravity) in all their effectiveness in the essential oils extracted from the rosemary leave (*R. officinalis*)The MHG method shows good advantages compared with traditional alternatives with less time of extraction; increased antimicrobial and antioxidant activities, spending just 15 minutes versus 2 hours for HD, cleaner features (no residue generation and no water or solvent used), and environmental impact (energy cost is fairly higher to perform HD than that required for rapid MHG isolation). (Farhat et al., 2011) studied the microwave steam diffusion (MSDf) utilizing microwave heating process to extract the essential oils from orange peel., and it is an advanced Steam Diffusion (SDF) technique. Same aromatic profile and yield for the essential oil extracted by SDF for 40 minutes and MSDf for 12 minutes.

2.5.2. Solvent Extraction

2.5.2.1. Solvent

Some delicate or fragile flower material, that is not tolerant for heating of the steam distillation, so conventional solvent extraction used for them. Different solvents including hexane, acetone, methanol, ethanol or petroleum ether, can be used for extraction (Areias et al, 2000; Pizzale et al., 2002; Kosar et al., 2005). To extract the oil; the plant material and solvent are mixing together, then exposure to heating then filtration, then evaporation. The extracted concentration (that consists of essential oils, wax, and fragrance) mixed with pure alcohol then distilled at low temperatures.the aromatic absolute oil has remained while alcohol absorbs the fragrance, then it is evaporated. Comparing with other methods This method makes the oil more expensive because it is a relatively time-consuming process. (Li et al, 2009). Th main components are phenolic (48.0%), thymol (3.4%) and carvacrol (44.6%).

2.5.2.2. Supercritical Carbon Dioxide

Some disadvantages are found in some traditional methods of extraction including steam distillation and solvent extraction and like a large number of organic solvents and long preparation time (Deng et al., 2005). İn addition to, losses of some volatile components, low extraction efficiency, degradation of the unsaturated components, and residue of the toxic solvent in the extract may be encountered (Jimenez-Carmona et al., 1999; Gliˇsica´ et al., 2007; Gironi and Maschietti 2008). So, the supercritical fluids used to extract the essential oils as an alternative method and because of the critical conditions of Carbon dioxide (CO2) is considered as the most common method. (Hawthorne et al., 1993; Jimenez-Carmona et al., 1999; Senorans et al., 2000). CO2 can be used as a very safe and inert medium to extract the essential oils from the aromatic plants and that because of SO2 shifting from gas to a liquid under high-pressure condition, In the final product, no solvent residue remains given that the liquid CO2 simply reverts to gas and evaporates under normal pressure and atmospheric temperature) Hawthorne et al., 1993) reported that although high solubilities of the compounds of essential oil in supercritical CO2, the rates of extraction were comparatively slow with pure CO2; after 90 minutes, the recovery

was 80%. Nevertheless, by the combination methods, high recoveries could be yielded through15-min static extraction with methylene chloride as a modifier followed by a 15-min dynamic extraction with pure CO2. The extraction efficiency was equivalent to HD performed for 4 h. The volatile components such as monoterpenes can be collected from the Supercritical Fluid Extraction (SFE) effluent by >90%. SFE was able to recover some organic components that were not extracted by HD (Hawthorne et al., 1993). Pereira and Meireles (2007) showed that supercritical fluid extraction is economically viable comparing with steam distillation. All that because of the lower yield and higher energy consumption.

2.5.2.3. Subcritical Water

Water under 100-374 °C and pressure maintain liquid water state has been introduced as an extractant. The efficiency of continuous subcritical water extraction was five times greater than the efficiency of the HD method, which associated with the essential oil's volume /1 g of the aromatic plants (Jimenez-Carmona et al., 1999). This subcritical water method produces more valuable essential oil and saves considerable costs associated with plant material and energy. A Study on the subcritical water extraction of lactones from a kava (Piper methysticum) root, compared to Soxhlet extraction with water, presented the following results; When extraction carried out at 175 °C, it lasted 20 minutes, while it takes 2 hours at 100 °C when extracted from ground samples with subcritical water, low yield was shown by 40% to 60% when the boiling for 2 hours and extraction with Soxhlet apparatus for 6 hours compared with that obtained using subcritical water method, all that during studying (Kubatova et al., 2001). Boiling for 2 h showed the lower yields by compared with that obtained using subcritical water.

2.5.2.4. Solvent-Free Microwave

Traditional methods as solvent or hydro diffusion extraction have some disadvantages such as losses of some volatile compounds, long extraction time, low extraction efficiency, , toxic solvent residue in the extract, and deterioration of unsaturated or ester components through thermal or hydrolytic effects (Pollien et al., 1998; Luque de Castro et al., 1999). This led to the consideration of using SFME. It

is a rapid extraction of essential oils from herbs, dry seeds, and aromatic spices. Many advantages belong to SFME method like environmental, shorter time, selectivity, and involving higher yield (Lopez-Avila et al., 1994; Tomaniova et al., 1998). SFME is a combination of microwave heating and dry distillation, performed at atmospheric pressure without any water or solvent. In addition, isolation and concentration of volatile components are performed in one stage (Lucchesi et al., 2004; Bayramoglu an et al., 2008). The higher essential oil yield of 0.054 ml/g was produced by SFME comparing with 0.048 ml/g produced by HD from oregano (Bayramoglu et al., 2008). Process time was decreased to 80% compared with conventional, and when microwave power at 662 W applying SFME method. Important advantages were obtained using microwave method comparing with conventional alternative like; higher yield (0.24% compared with 0.21% for HD and 0.05% for CP), shorter extraction times (30 minutes compared with 3 h by HD and 1 h by Cold Pressing [CP]), cleaner features as no residue generation and no water or solvent used, high environmental impact and antimicrobial activities. Moreover, the cost of energy is appreciably higher for performing HD and for mechanical motors (CP) than that required for rapid microwave extraction (Ferhat et al., 2007).

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.1. Plant Materials

Thymbra spicata (thyme), *Pimpinella anisum* (anise), *Folium menthae* (mint), *Matricaria chamomilla* (chamomile) and *Ocimum basilicum* (Basil) were obtained during June to July in 2014 from Turkey (Gaziantep) and Syria (Aleppo and Idleb). Each of the plants was obtained as dry form. They did not exposure to sunlight to avoid loss of active components.

3.1.2. Microbial Strain

The essential oils were assayed against following microorganisms: *Escherichia coli* KUEN 1504, *Salmonellaenterica* subsp. *enterica* ser. TyphimuriumKUEN 1357, *Yersinia enterocolitica* 03, *Pseudomonas aeruginosa* ATCC 39327, *Listeria monocytogenes* ATCC 35152, *Shigella dysanteria* ATCC *11835*, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* KUEN 8, *Bacillus subtilis* ATCC 13933, *Klebsiella pneumoniae* ATCC 35657, *Enterococcus faecalis* ATCC 29212, *Streptococcus thermophiles* ATCC 14485, *Saccharomyces cerevisiae* ATCC 561, *Candida rugosa* ATCC 10571, *Aspergillus flavus*ATCC 11498, *Aspergillus ochraceus* 151, *Aspergillus niger* ATCC 9142, *Penicillium expansum* ATCC117, *Rhizopus oryzae* ATCC 4858 and *Fusarium graminearium* ATCC 46779. *Escherichia coli* KUEN 1504, *B. cereus* KUEN 8 and *S.enterica* subsp. *enterica* ser. Typhimurium KUEN 1357 were obtained from Microorganism's Culture Collection Center, Faculty of Medicine, University of İstanbul, İstanbul Turkey. *Y. enterocolitica* 03 was obtained from the Department of Microbiology, Gülhane Military Faculty, Ankara, Turkey. *A. ochraceus* 151was

obtained from USAMV (University of Agronomic Science and Veterinary Medicine, Bucharest, Romania.All ATCC cultures were obtained from American type culture collection. Stock cultures of all strains were maintained on Brain heart infusion agar (BHIA, Oxoid CM1, England) slants at 4°C.

3.1.3. Media and Chemicals Used

Tween 80 (Merck), dimethylsulfoxide (Sigma, dortet,UK), brain heart infusion agar (BHIA; Oxoid CM1, England), brain heart infusion broth (BHIB; Oxoid CM1, England), Sabouraud's dextrose agar (SDA; Difco, Detroit, USA), peptone (Oxoid CM1, England).

3.2. Methods

3.2.1. Preparation of Stock Solutions

DMSO-Essential oil solution: suspension of the essential oil was done in di-methyl sulphoxide (DMSO) (Sigma, Dortet, UK) and used as an emulsifier before adding the mixture to the antimicrobial test media. A stock solution of 75 % essential oil concentration was prepared by adding 7.5 ml of essential oil into 2.5 ml of DMSO that was used as stock solution. An amount of DMSO + essential oil mixture was diluted from 0.1 to 5 µg/ml using BHIB that was used as stock solution-2. Weighing of 10 g of sodium hydroxide (NaOH) then dissolving in 250 ml of distilled water. H_2O_4 solution (25 %): 95 % (v/v) H_2SO_4 solution was diluted with distilled water to obtain 25 % (v/v) H_2SO_4 solution.

3.2.2. Sterilization

The media and solutions used in the experiments had been sterilized in an autoclave at 121^{o}C for 15 min. All glass wares were sterilized in a dry air oven at 180^{o}C for 2 h.

3.2.3. Essential Oil Extraction

Air dried parts of plants (leaves and flowers) were ground in a mechanical grinder to increase the efficiency of the extraction and thereby increasing the yield to be obtained. The essential oils were extracted related to the amount of the plant material available, and the method that used was hydro-distillation for three hours by using a Clevenger-type apparatus Figure 3.1. The species used to extract the essential oil were those of thyme, anise, basil, mint, and chamomile. The extraction of the essential oils was done via Clevenger hydrodistillation method. (Anonymousa, 1996; Boundtilrou et al., 2007). Essential oil (EO) was obtained from the leaves and flowers of dried plants. Ground plant materials were placed in a clavenger vessel (Figure 3.1.) with double distilled water in 1:2 (Material:Water) ratio through opening and hydrodistilled for three hours. The heating mantel was switched on, and the extraction process was set to start. Collection of essential oils was done through condensing of the extract in the cooling vapor and reading the quantity of the essential oil was done directly from the graduated column, then decanting off them into the bottles for storage. Then the process repeated again with a new fresh set of leaves and so on. The extracted essential oils kept in small (10 ml) sterile dark bottles at 4°C until used. The essential oils were then used for antimicrobial tests in the research laboratory of the Department of Food Engineering (Gaziantep University, Gaziantep, Turkey).

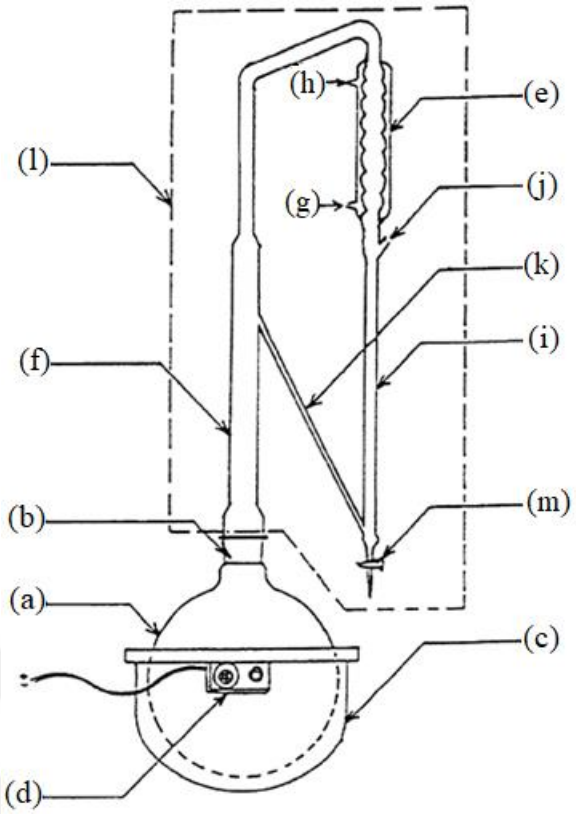

Figure 3.1. Clavenger

Clavenger is a laboratory apparatus that has been using for the distillation of essential oils as shown in Figure 3.1. The apparatus (I) is attached to the spherical glass vessel (a) adding the plant material through the water 1:2 ration by opening (b) that used to throw the discharging mixture after the process is finished. All this system will be put on a heater (c), that has an energy regulator (d) for setting the temperature. The plant material begins boiling after a few minutes. The vapors so formed are passed through a vertical condenser (e) through a long vertical glass tube (f). The cold water around the condenser tube is circulated through inlet (g) and outlet (h). The condensed distillate gets collected in a measuring tube (i) connected to the outlet of the condenser where an air outlet (j) open to atmosphere is placed. The volatile oil separates as an upper layer, from the distillate because of its density difference, as the oil is lighter than the water. A return tube (k), for recycling of aqueous part of the distillate, connects the bottom of the measuring tube (i) and vertical tube (f). The oil is collected at the outlet by opening the Stop-cock valve (m).

3.2.4. Stock Culture Preparation

Microorganisms inoculated were prepared by growing the cells in 9.0 ml of BHIB for 24 hours at 37°C. Approximately 0.1 ml of culture was shifted to 9.0 ml of BHIB at 2 consecutive 24 hours intervals immediately before each experiment. Diluting the bacterial suspension by adding it to the peptone water for providing initial cell counts which are about 10^9 Colony Forming Unit (cfu/ml) according to the method that called the surface plate counting. (Erkmen, 2015). The working bacterial suspension was diluted with the appropriate BHIB to obtain 10^7 cfu/ml. The harvesting of molds' spores was done by flooding the PDA agar slant culture surface with sterile cold 0.85% NaCl water and the removing was done by scraping with a sterile glass rod. The suspension was centrifuged at 1,600g for 15min (centrifuge 5810 R, Eppendorf, Hamburg, Germany) and twice washing was done in cold 0.85% NaCl water, then resuspension was done with PDB and then storage at 4°C. The stock mold suspensions contained about 10^8 mold spores/ml. The working mold spore suspension was diluted with the appropriate PDB to obtain 10^5 mold spores/ml and 10^6 yeast cells/ml. Resulting microbial suspensions were used as inocula in the antimicrobial activity tests (Erkmen, 2013).

3.2.5. Antibacterial Activity

The broth microdilution method was performed to indicate antimicrobial impact and activities (MTC, MIC MBC, and MFC) of essential oil that obtained from the aromatic plants (Giweli et al., 2012; Thosar et al., 2013; Seyyednejad et al., 2014). The essential oils were suspended in dimethylsulphoxide and were used as an emulsifier before adding the mixture the cell suspension. The used concentration of DMSO (< 0.15 % v/v) was tested against microorganisms and there was no observation of the antimicrobial activities. The mixture from essential oils and DMSO was added into 5 ml of test tubes containing 1.5 ml of BHIB to provide the required amount of essential oil concentration in 2 ml of test medium BHIB. After adding the essential oil-DMSO mixture, test medium BHIB was completed to 1.9 ml by adding the required amount of BHIB. The evaluation of the antimicrobial activity of five essential oils that obtained from the aromatic plants against each of the twelve different tested bacteria and eight different tested fungi (mold and yeast), 100 µl at

each working culture of one test pathogen was inoculated into 2 ml of each treatment tube, separately. Therefore, the final volume of the test medium was 2.0 ml. Final essential oil concentrations in the test medium were between0.1 to 200 µg/ml. Final bacterium count in tubes was about 10^6 cfu/ml. The final mold spores and yeast in tubes were 10^5 cfu/ml, the tubes were vortexed to mix suspension and incubation was done at 35°C for the period from 24 to 48 h in a water bath (ST-402; Nüve, Sanayii ve Malzemeleri İmalat ve Ticaret A.Ş., İstanbul, Turkey). The water bath was shacked at 20 rpm. After incubation, following antimicrobial activities were designated fromtubes. (i) When microbial growth does occur at an essential oil concentration (appearance of turbidity) before the concentration without growth, this was indicated as the essential oil concentration inadequate to kill the microorganism and was designated as maximum tolerance concentration (MTC, µg/ml). Therefore, MTC which is the highest concentration of essential oil, that has no effect on the bacterial growth (turbidity; allowed growth) in the tube. (ii) The lowest essential oil concentration which microbial growth does not occur (without turbidity) in the tube was designated as minimum inhibitory concentration (MIC, µg/ml). (iii) For minimum bactericidal biocidal concentration (MBC, µg/ml), subcultures were done starting from MIC tube to next three tubes without turbidity by streak plating onto BHIA. The incubation of BHIA plates was done at 37°C for 18‑ 20 hours on the next day, the readings were taken. For minimum fungi concentration (MFC), subcultures were done on SAD plates as done for MBC procedure. The incubation of SAD plates was done at 25^{o}C for one to three days and after incubation, and readings were taken from 1to3 days. Control broths with used DMSO (25 % v/v) and essential oil in 2 ml BHIB tubes without microbial culture inoculation were used to check the microbial contamination from the essential oil and DMSO. All experiments were duplicated and the experiments against each organism with each essential oil were repeated twice.

3.3. Statistical Analysis

The standard deviations and average of MTC, MIC, MBC, and MFC values were estimated. Comparing the mean of MTC, MIC, MBC and MFC values (µg/ml) between different groups happened through Independents Samples t-test and One-Way ANOVA with the least significant differences procedure at a significance level

of 0.05. The analyses of the statistical were implemented with IBM SPSS v22 (2013; IBM Corporation) and these statistical tests were employed to detect statistically significant differences at the 95% significance level ($p<0.05$).

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Results

In this thesis study, MIC, MBC, (MFC for fungi) and MTC measurements of the essential oils that extracted from the aromatic plants that collected from Syria and Turkey had been examined against the studied organisms; bacteria (both Gram-negative bacteria and Gram-positive bacteria), yeasts, and molds. Growth in tubes containing BHIA with the used DMSO (Dimethyl sulfoxide) concentration (< 0.15 % v/v) were not observed. The static concentration of essential oil was the lowest concentration that microorganisms did not grow in BHIB, but microorganisms grew on BHIA. The cidal concentration was the lowest concentration that microorganism did not grow in BHIB and BHIA. The following findings are resulted by the effect of Syrian and Turkish plant essential oils that extracted from five types of Syrian aromatic plants and five types of Turkish aromatic plants; *Thymbra spicata* (thyme), *Pimpinella anisum* (anise), *Folium menthae* (mint), *Matricaria chamomilla* (chamomile), and *Ocimum basilicum* (Basil)) on the Gram-negative bacteria and Gram-positive bacteria (*E. coli*, *S.* Typhimurium, *Y. enterocolitica*, *P. aeroginesa*, *S. dysenteriae*, *K. pneumoniae*, *L. monocytogenes*, *S. aureus*, *B. cereus*, *B. subtilis*, *E. faecalis*, and *S. thermophilus*), molds (*A. flavus*, *A. ochraceus*, *A. niger*, *P. expansum*, *R. oryzae*, and *F. graminearum*), and yeasts (*S. cerevisiae* and *C. rugosa*).

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that can inhibit the visible growth of a microorganism after incubation and it is important in diagnostic laboratories to determine the potency of new antimicrobial agents and also to confirm the resistance of microorganisms to an antimicrobial agent. MIC is usually regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism.

Minimum Bactericidal Concentration (MBC); It is considered as complementary to the MIC, lowest concentration of an antimicrobial that will kill particular bacteria, wheres the MIC is the lowest level of antimicrobial agent that greatly inhibits growth; the MBC demonstrates the lowest level of an antimicrobial agent resulting in microbial death, that means; if MIC shows inhibition, plating the bacteria onto agar might still result in microorganism growth because the antimicrobial did not cause death.

Maximum Tolerance Concentration (MTC): When microbial growth does occur at an essential oil concentration before the concentration can cause inhibition, this was indicated as the µg/ml. So, MTC is the highest concentration of essential oil that has no effect on the growth of the test microorganism. MIC is the minimum effective dose against the microorganism to inhibit the growth while MTC is the dose which is tolerable to the desired species. The MIC may be used against microorganisms to kill them in minimum dose but because every antimicrobial agent is toxic to the host too so MIC does not only damage microbes but also to the host taking it. MTC is so the maximum dose tolerable to the host. An ideal antimicrobial agent should have minimum MIC and higher MTC. The ratio of MTC versus MIC is a measurement of the therapeutic index to know how high the effectiveness of an antimicrobial. MIC is the lowest concentration of the inhibitor that produces the complete absence of growth of test microorganism after sufficient incubation. MTC is the concentration at which there is no difference in the growth when compared to the untreated/control sample.

4.1.1. Antimicrobial effect of plant essential oils from Turkey and Syria on bacteria

4.1.1.1. Antimicrobial effect of *Thymbra spicata* (thyme) essential oil on Gram-negative bacteria

The antimicrobial values; MIC, MBC, and MTC of Syrian and Turkish thyme against the Gram-negative bacteria are shown in Figures 4.1 and 4.2 respectively; where the MIC values for essential oils that obtained from Syrian thyme were found as 0.25 µg/ml on *P. aeruginosa, S. dysenteriae, S.* Typhimuriumand *Y. enterocolitica*, as 0.5 µg/ml on *E. coli* and; 1.25 µg/ml against *K. pneumoniae* which are ranged between 0.25 and 1.25 µg/ml on Gram-negative bacteria. So, the Syrian

and Turkish essential oil has the same impact against *S.*Typhimurium, *P. aeruginosa, S. dysenteriae*, and *K. pneumoniae*. The Turkish thyme is more effective than the Syrian one against *Y. enterocolitica* and *E. coli* with low concentration of essential oils; These results are not similar with (Giweli et al., 2011) where they mentioned that values of MIC against *E. coli* were ranged from 0.0005 and 0.015 µg/ml and against the *S*. Typhimurium were ranged from 0.1 and 0.001 µg/ml; and that because of the difference of essential oil's compounds and regions. However results from (Giweli et al., 2011) were agreed with the MIC against *P. aeruginosa*. Also, the results for *E. coli* with MIC values from Turkey were 0.25 and from Syria were 0.5 µg/ml ; were different from Burt (2004) results. On the other hand, (Markovic et al., 2011) found that the values of MIC against *E. coli* ranged between (0.3 and 5 µg/ml) it was agreed with MIC values that obtained from Syrian but not with the Turkish ones. Comparing with the MIC's values against *P. aeruginosa* and *S.*Typhimurium did not meet with their results that were ranged between 0.5 and 1.6 µg/ml and 0.3 and 1.25 µg/ml, respectively, which may belong to the components of essential oil and to the difference in their activities. The MBC values for essential oils obtained from Turkish thyme were ranged from the lowest level; 0.25 µg/ml against *P. aeruginosa, Y. enterocolitica* and *S. dysenteriae* to the highest level; 1.5 µg/ml on *K. pneumoniae*. The MBC values for essential oils obtained from Syrian thyme were ranged from the lowest level; 0.5 µg/ml against *P. aeruginosa*, *Y. Enterocolitic* and *S. dysenteriae* to the highest level; 1.75 µg/ml on *K. pneumoniae*. The Syrian and Turkish thyme have the same impact against *E. coli*, but Turkish essential oil has more impact than Syrian against the rest of Gram-negative bacteria with a low concentration of MBC. Comparing these results with (Giweli et al., 2011), MBC values seem to be very different in the range. On the opposite these results are similar with the (Markovic et al., 2011) where MBC values against *E. coli* (were between0.5 to 10 µg/ml) and *S.*Typhimurium (were between 0.5 to 5 µg/ml); also did not agree with MIC's values against *P. aeruginosa* that were between0.6 to 3.1 µg/ml. The MTC values for essential oils obtained from Syrian thyme were between 0.1 against *S*. Typhimurium, *Y. enterocolitica, P. aeruginosa* and *S. dysenteriae*, and 1 µg/ml against *K. pneumoniae*. On the other hand, MTC values for Turkish thyme were lowest (0.05 µg/ml) against *Y. enterocolitica*, and highest (1 µg/ml) against *K. pneumoniae*. So, the Syrian and Turkish thyme essential oils have the same antimicrobial effects on *K. pneumoniae*, *S.*Typhimurium, and *S. dysenteriae*. But

generally, the most resistant bacterium was *K. pneumoniae* among Gram-negative bacteria.

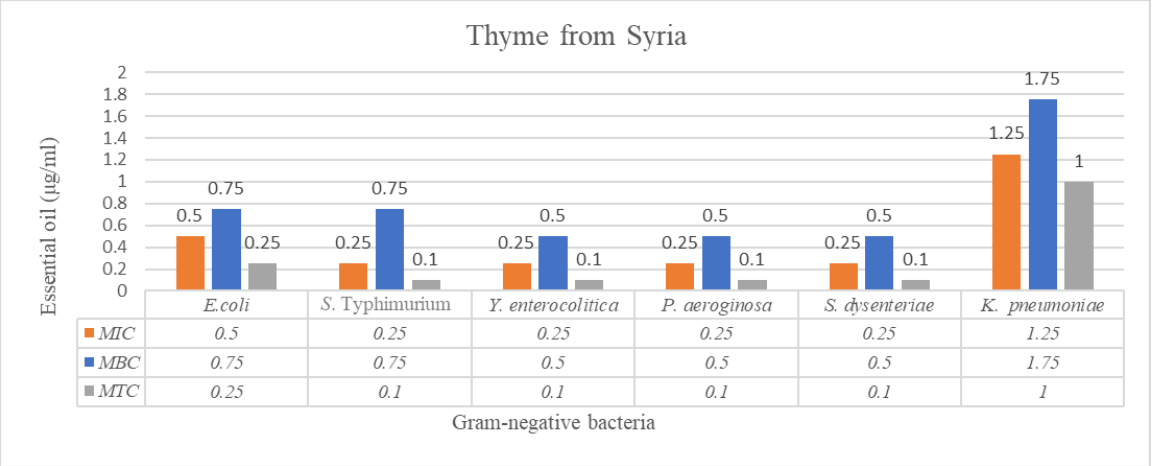

Figure 4.1. Antimicrobial values (MIC, MBC and MTC) of the Syrian thyme against the Gram-negative bacteria

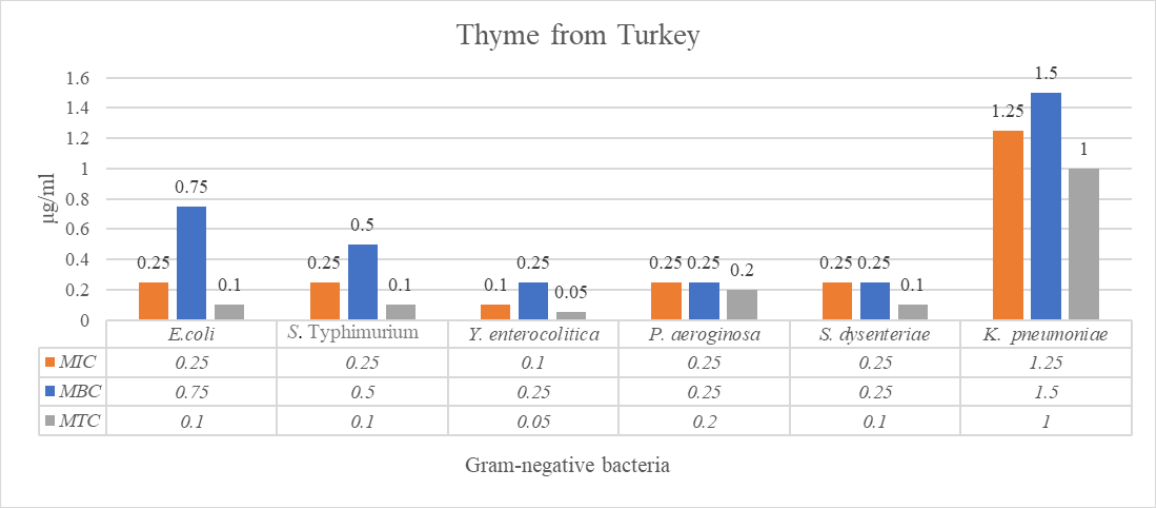

Figure 4.2. Antimicrobial values (MIC, MBC and MTC) of the Turkey thyme against the Gram-negative bacteria

4.1.1.2. Antimicrobial effect of *Thymbra spicata* (thyme) essential oil on Gram-positive bacteria

The antimicrobial values of MIC, MBC and MTC of the Syrian and Turkish thyme against the Gram-positive bacteria are given in the Figures 4.3 and 4.4 respectively; where the MIC values for essential oils that obtained from Syrian thyme were between the lowest level; 0.25 µg/ml against *S. thermophilus* and the highest level; 1.25 µg/ml on *L. monocytogenes*; and the MIC values for essential oils that obtained from Turkish thyme were at the lowest level; 0.25 µg/ml on *S. aureus* and *B. subtilis*

and the highest level; 0.75 µg/ml against *L. monocytogenes* and *E. faecalis*. So, Turkish and Syrian thyme have the same impact against *E. faecalis*. But the Syrian thyme has impact more than Turkish one against *S. thermophilus* with low values of MIC and the Turkish thyme has lower MIC values against all Gram-positive bacteria other than Syrian thyme. For Gram-positive bacteria (Giweli et at., 2011) found the MIC's values against *S. aureus* were ranged from 0.001 and 0.05 µg/ml, and 0.0005 and 0.05 µg/ml and against the *B. subtilis* had dramatically lower than results obtained in this study. However, (Markovic et al., 2011) published results which were similar with these results; where their MIC values were between 0.25 and 3.1 µg/ml; especially in the Turkish essential oil ranged from 0.25 to 0.75 µg/ml. The MBC values for essential oils obtained from Syrian thyme were ranged from 0.5 µg/ml to 1.25 µg/ml. These results are very close to the MBC values for essential oils that obtained from Turkish thyme were at the lowest level 0.5 µg/ml on *S. aureus* and *B. subtilis*, and the highest level 1 µg/ml on *L. monocytogenes*, *E. faecalis* and *B. cereus*. So, the Syrian essential oil is better than Turkish one against the *S. thermophilus* with low concentration but the Turkish essential one is better than Syrian against the rest of studied Gram-positive bacteria. For MBC results; (Markovic et al., 2011) found similar MBC values against *S. aureus* ranged between 0.5 and 6.25 µg/ml and the Turkish essential oil were closer than the Syrian. They also reported MBC values and against *B. cereus* from 0.25 to 2.5 µg/ml and against *E. faecalis* from 0.5 to 10 µg/ml. The MTC values for essential oils that obtained from Syrian thyme were ranged between 0.1 µg/ml against *S. thermophilus* and 1 µg/ml against *L. monocytogenes*, which is similar with the MTC values for essential oils that obtained from Turkish thyme is 0.1 µg/ml on *S. thermophilus*, *S. aureus* and *B. subtilis* and 0.5 µg/ml on *E. faecalis* and *L. monocytogenes*. The Syrian and Turkish thyme have the same impact against *S. thermophilus* and *E. faecalis*, but the Turkish thyme has more impact that Syrian against the rest of studied positive bacteria atlow values of MTC.

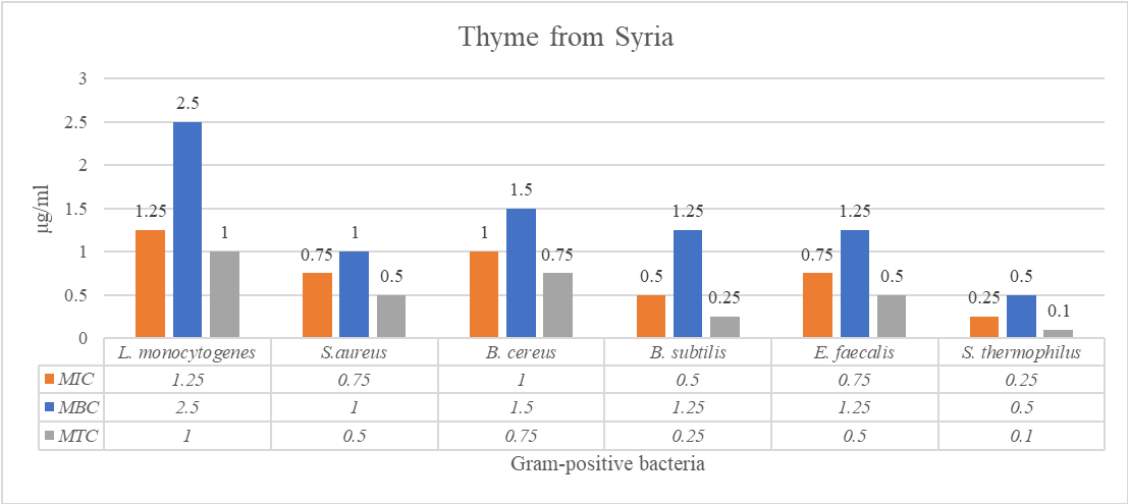

Figure 4.3. Antimicrobial values (MIC, MBC and MTC) of the Syrian thyme essential oil against the Gram-positive bacteria

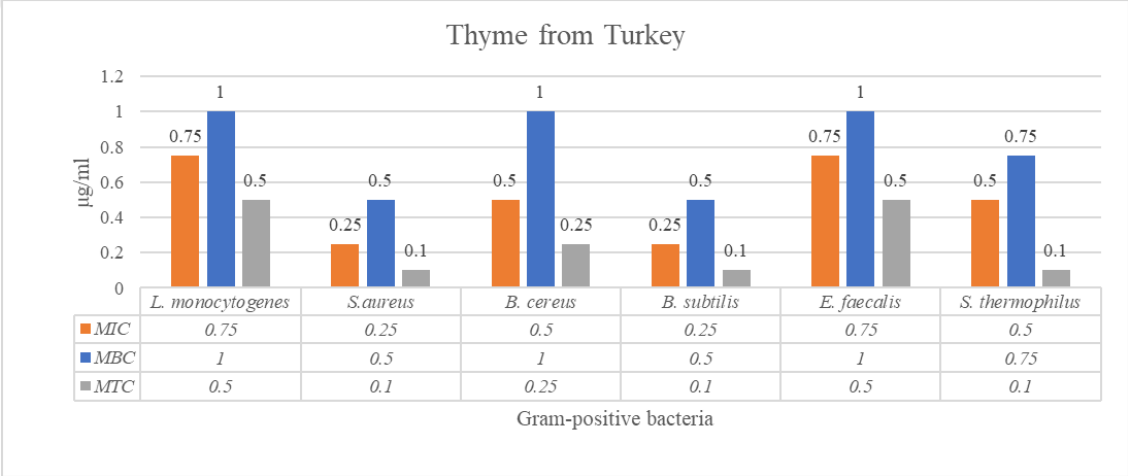

Figure 4.4. Antimicrobial values (MIC, MBC and MTC) of the Turkish thyme essential oil against the Gram-positive bacteria

4.1.1.3. Antimicrobial effect of essential oil *Pimpinella anisum*(anise) on Gram-negative bacteria

The antimicrobial values (MIC, MBC, and MTC) of the Syrian and Turkish anise against Gram-negative bacteria are shown in Figures 4.5 and 4.6 respectively, where the MIC values for essential oils that obtained from Syrian anise were ranged between 20 µg/ml on *Y. enterocolitica*, and 60 µg/ml against *K. pneumoniae*. MIC values for essential oils obtained from Turkish anise were ranged between 10 µg/ml against *E. coli* and *Y. enterocolitica*, and 45 µg/ml against *S. dysanteriae*. So, the Turkish anise is more effective than the Syrian one with low concentration against

Gram-negative bacteria. These results did not match with the results done by (Gumus et al., 2016) where the MIC's values for the anise against *S. aureus* and *E. coli* were 500 µg/ml. The MBC values for essential oils obtained from Syrian anise were between 20 µg/ml against *Y. enterocolitica* and 80 µg/ml against *K. pneumoniae* did not meet with the MBC values for essential oils obtained from Turkish anise that ranged between 10 µg/ml against *Y. enterocolitica* and 75 µg/ml on *K. pneumoniae*. So, one can say that the Turkish anise essential oil with low concentration is more effective than Syrian ones against all the studied Gram-negative bacteria. The MTC values for anise essential oils obtained from Syria were ranged between 15 µg/ml against *Y. enterocolitica* and 45 µg/ml against *S. dysanteriae* and *K. pneumoniae* which did not align with the MTC values for essential oils that obtained from Turkish anise that ranged between 7.5 µg/ml against *E. coli* and *Y. enterocolitica* and 45 µg/ml against *K. pneumoniae*; the Syrian and Turkish have the same impact against the *K. pneumoniae* but the Turkish ones have more impact than Syrian against the rest of the studied Gram-negative bacteria with low values of MTC. So, the most resistant bacteria are found as *S. dysanteriae* and *K. pneumoniae* for both Turkish and Syrian anise essential oil.

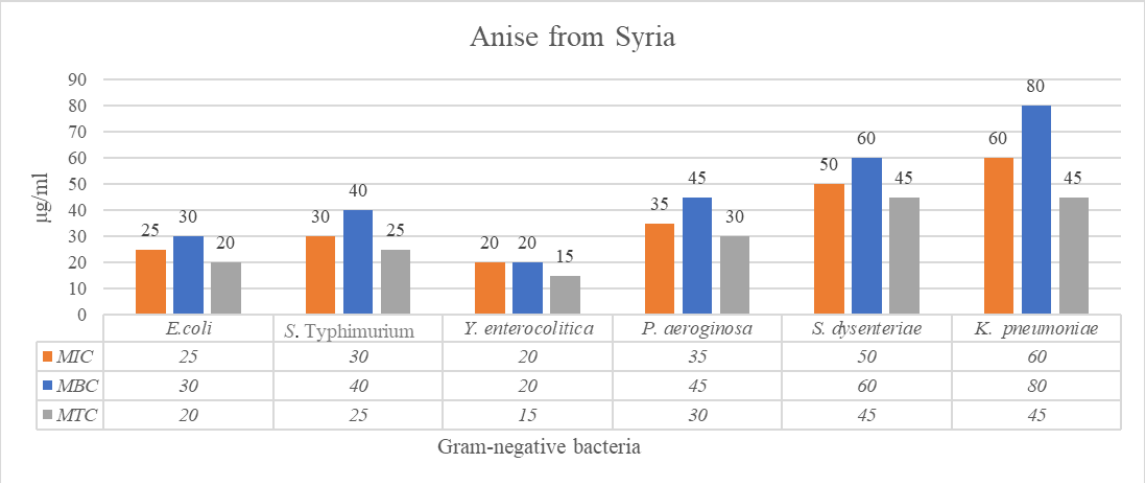

Figure 4.5. Antimicrobial values (MIC, MBC and MTC) of Syrian Anise essential oil against the Gram-negative-bacteria

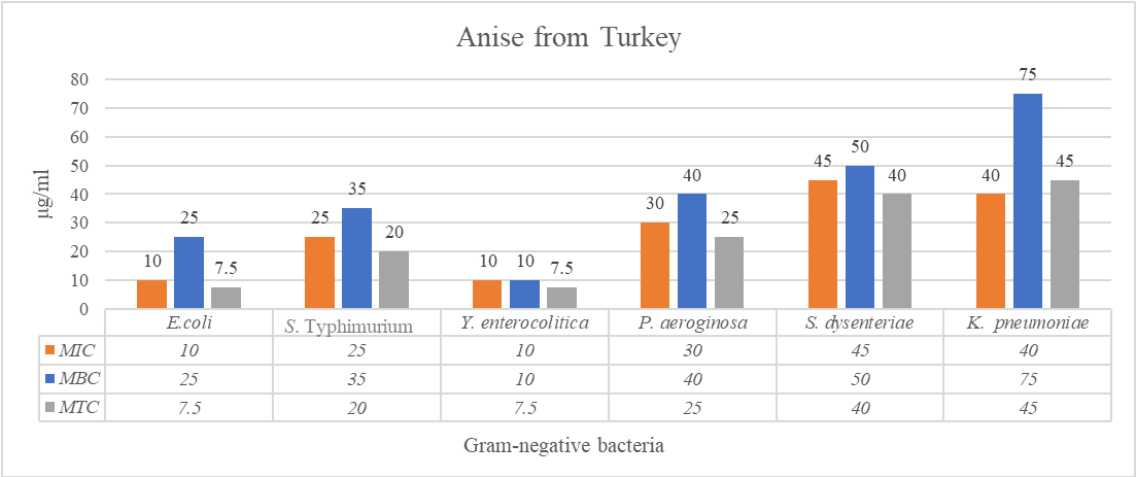

Figure 4.6. Antimicrobial values (MIC, MBC, and MTC) of Turkish anise essential oil on the Gram-negative bacteria

4.1.1.4. Antimicrobial effect of essential oil *Pimpinella anisum*(anise) on Gram-positive bacteria

The antimicrobial values (MIC, MBC, and MTC) of the Syrian and Turkish anise against the Gram-positive bacteria are given in Figures 4.7. and 4.8 respectively. The essential oil MIC values obtained from Syrian anise were 10 µg/ml against *S. aureus* and 70 µg/ml against *B. cereus*. The MIC values for essential oils obtained from Turkish anise were ranged between 5 µg/ml on *S. aureus* and 50 µg/ml on *B. cereus*. Turkish anise has lower MIC values than Syrian anise it can be said more effective than Syrian anise on Gram-positive bacteria. The MBC values for essential oils obtained from Syrian anise were ranged between 15 µg/ml against *S. aureus*, and 80 µg/ml against *B. cereus* similar with the MBC values for essential oils that obtained from Turkish anise was the lowest level 10 µg/ml on *S. aureus* and the highest level 65 µg/ml on *B. cereus*. So, the Syrian and Turkish essential oil has the same impact against the *L. monocytogenes*, but Turkish anise essential oil has more effective than Syrian one against the rest of microorganisms with low values of MBC. The MTC values for essential oils obtained from Syrian anise were ranged between 5 µg/ml against *S. aureus* and the highest level 55 µg/ml against *B. cereus*. For Turkish anise, MTC values were 2.5 µg/ml against *S. aureus* and 45 µg/ml against *B. cereus*. So, the Turkish anise essential oil is more effective than Syrian ones against all the Gram-positive bacteria with low values of MTC.

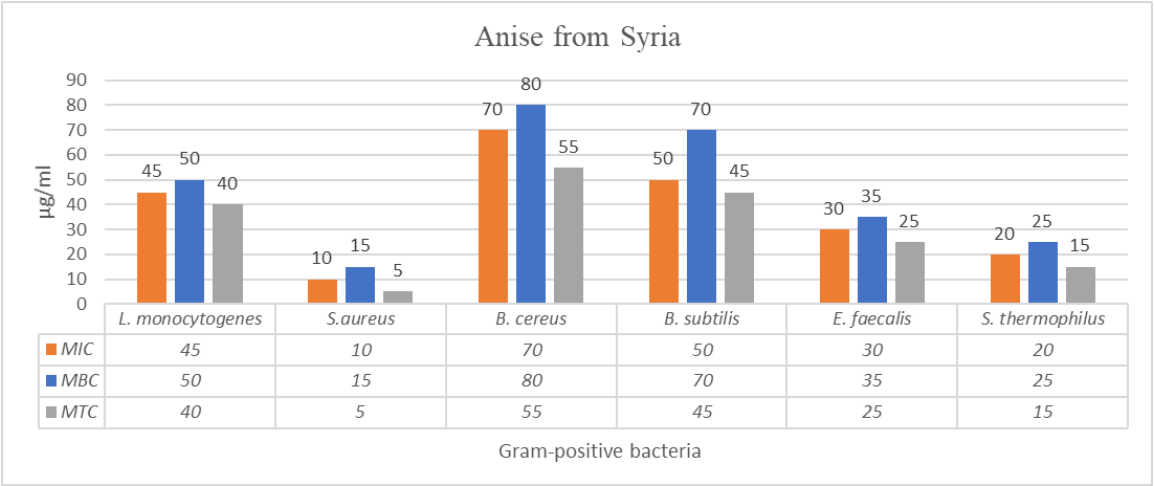

Figure 4.7. Antimicrobial values (MIC, MMC and MTC) of the Syrian anise essential oil against the Gram-positive bacteria

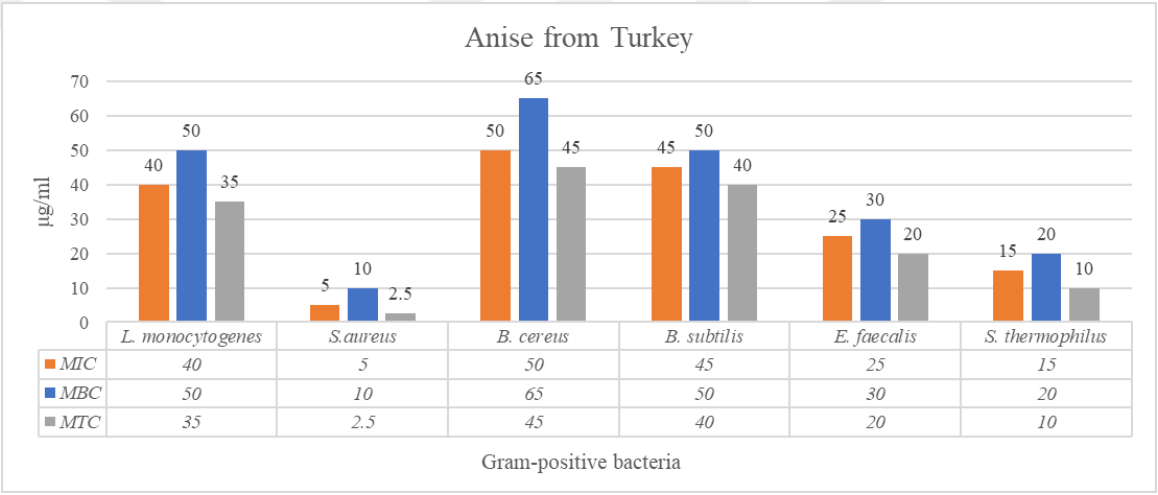

Figure 4.8. Antimicrobial values (MIC, MBC and MTC) of Turkish anise essential oil against the Gram-positive bacteria

As a brief summary MIC, MBC and MTC values of Turkish and Syrian anise follow a parallel trend within each other. While MIC values are increasing in order among selected bacteria, MBC and MTC values are also increasing with the same order. According to this result, the most resistant Gram-positive bacteria seems to be *B. cereus* against both Turkish and Syrian anise essential oils.

4.1.1.5. Antimicrobial effect of *Folium menthae* (mint) essential oil on Gram-negative bacteria

The antimicrobial values of MIC, MBC, and MTC of the Syrian and Turkish mint against the Gram-negative bacteria are given in Figures 4.9 and 4.10 respectively. MIC values for essential oils obtained from Syrian mint were ranged between 20

µg/ml on *E. coli* and 90 µg/ml on *K. pneumoniae*. Comparing with the MIC values for essential oils that obtained from Turkish mint that ranged between 15 µg/ml on *P. aeroginosa* and 75 µg/ml on *K. pneumoniae*. The Syrian mint essential oil has lower MIC values than Turkish one against *E. coli*, but the Turkish mint essential oil has lower MIC values than the Syrian ones against the rest tested Gram-negative bacteria so, the Syrian ones have more impact than Turkish ones against *E. coli* and Turkish has more impact than Syrian against the rest. (Thosar et al., 2013) found the MIC values were 1 µg/ml against *E. coli*. The MBC values for essential oils obtained from Syrian mint were ranged between 25 µg/ml on *E. coli* and 100 µg/ml on *K. pneumoniae*. However, MBC values for Turkish mint ranged between 25 µg/ml on *P. aeruginosa* and *S. dysanteriae* and 85 µg/ml on *K. pneumoniae*. which did not agree with results that done by (Thosar et al., 2013) where the MBC values for mint essential oil were 4 µg/ml against *E. coli*.And the Syrian mint essential oil has more impact than Turkish against *E. coli*, and Turkish ones have more impact than Syrian against the rest of studied Gram-negative bacteria with low values of MBC. The MTC values for essential oils that obtained from Syrian mint were the lowest level 15 µg/ml against *E. coli* and the highest level 90 µg/ml against *K. pneumoniae*, and the MTC values for essential oils that obtained from Turkish mint were at the lowest level 10 µg/ml against *P. aeruginosa*, and the highest level; 65 µg/ml on *K. pneumoniae*. When the regional difference is examined, the Turkish mint essential oil has lower MTC values than Syrian ones, so the Turkish mint has impact more than Syrian ones except for *E. coli* with low values of MTC.

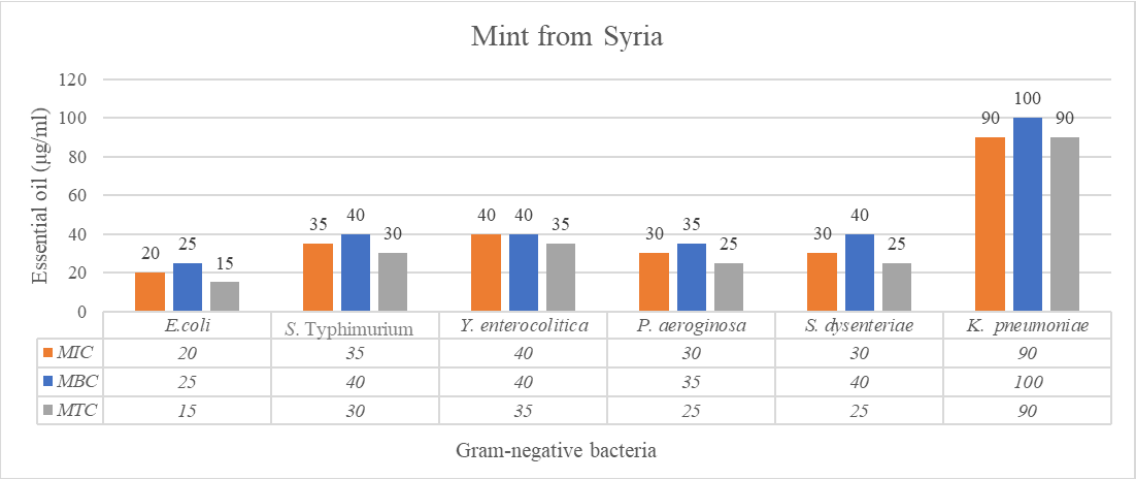

Figure 4.9. Antimicrobial values (MIC, MBC, and MTC) of the Syrian mint essential oil against the Gram-negative bacteria

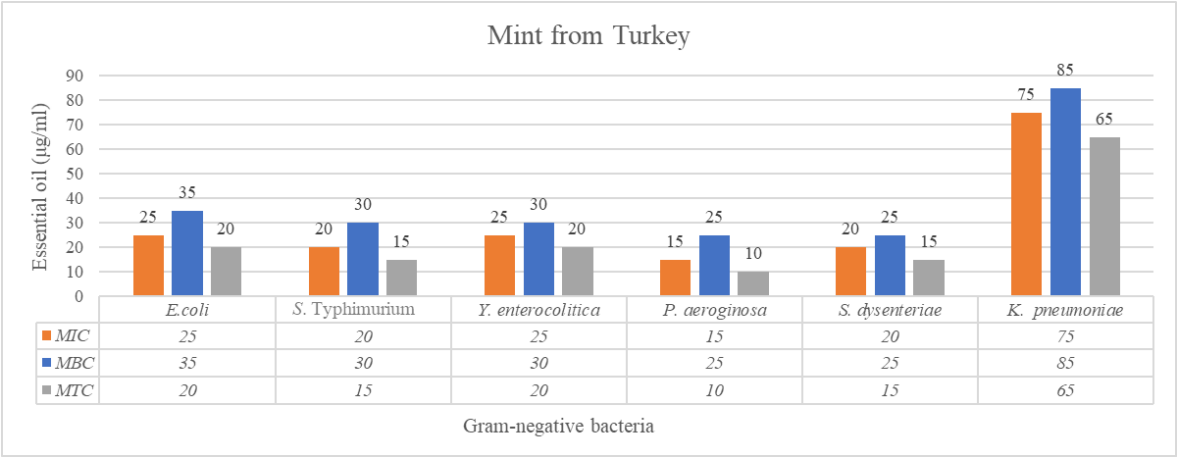

Figure 4.10. Antimicrobial values (MIC, MBC, and MTC) of the Turkish mint essential oil against the Gram-negative bacteria

4.1.1.6. Antimicrobial effect of *Folium menthae* (mint) essential oil on Gram-positive bacteria

The essential oils' antimicrobial values obtained from the Syrian and Turkish mint against Gram-positive bacteria are shown in Figures 4.11 and 4.12 respectively. The essential oil MIC values obtained from Syrian mint were at the lowest level; 20 µg/ml against *S. thermophilus* and the highest level; 40 µg/ml on *B. cereus* and *B. subtilis*. Similar results are obtained from Turkish mint where at the lowest level; 5 µg/ml on *S. thermophilus* and highest level; 30 µg/ml *B. cereus*. The Syrian and Turkish mint have the same impact against the *E. faecalis*, but every MIC value of Turkish mint is lower than Syrian mint MIC values so, the Turkish ones have more impact against the Syrian ones. According to results of (Gumus et al., 2016) MIC values for the anise against the *L. monocytogenes* and *S. aureus* were 125 µg/ml so they did not agree. In addition, that these results did not also agree with the findings that done by (Nilim Thosar et al., 2013) where the MIC values were 32 and 2 µg/ml against *E. faecalis*and *S. aureus* respectively. Our results are very different than results of (Basheer et al., 2013) where their results were 8 µg/ml, 64 µg/ml and 128 µg/ml against *S. aureus*, *E. coli*, *P. aeroginosa* respectively. The MBC values for essential oils that obtained from Syrian mint were at the lowest level; 30 µg/ml against *S. aureus* and *S. thermophilus* but the highest level; 45 µg/ml against *B. cereus* and *B. subtilis*. Comparing with the MBC values for essential oils obtained from Turkish mint were ranged between 20 µg/ml on *S. thermophilus* and 40 µg/ml on *B. cereus*. The Turkish mint has impact more than Syrian ones against all the

studied bacteria with low values of MBC and these results are not agreed with (Thosar et at., 2013); where they had found the MBC values of mint essential oil as 2 µg/ml against *S. aureus*; as 32 µg/ml against *E. faecalis*. The MTC values for Syrian mint were ranged between 15 µg/ml against *S. thermophilus* and 35 µg/ml against *B. cereus* and *B. subtilis*. On the other hand, Turkish mint MTC values ranged between 7.5 µg/ml against *S. thermophilus* and 20 µg/ml against *B. cereus* and *E. faecalis*. MTC of *E. faecalis* was same for both Syrian and Turkish mint, but all other MTC values were lower for Turkish mint than Syrian mint.

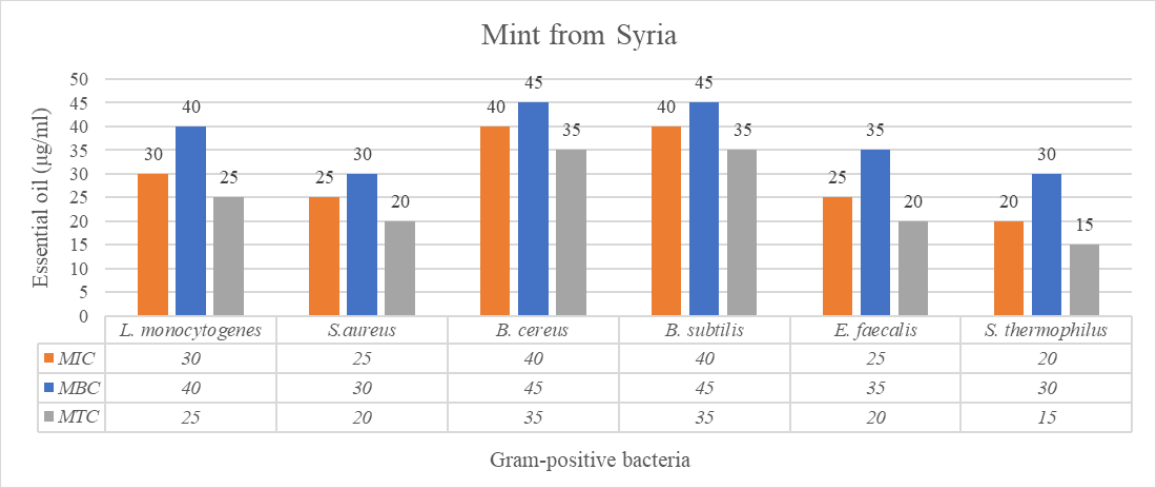

Figure 4.11. Antimicrobial values (MIC, MBC, and MTC) of the Syrian mint essential oil against the Gram-positive bacteria

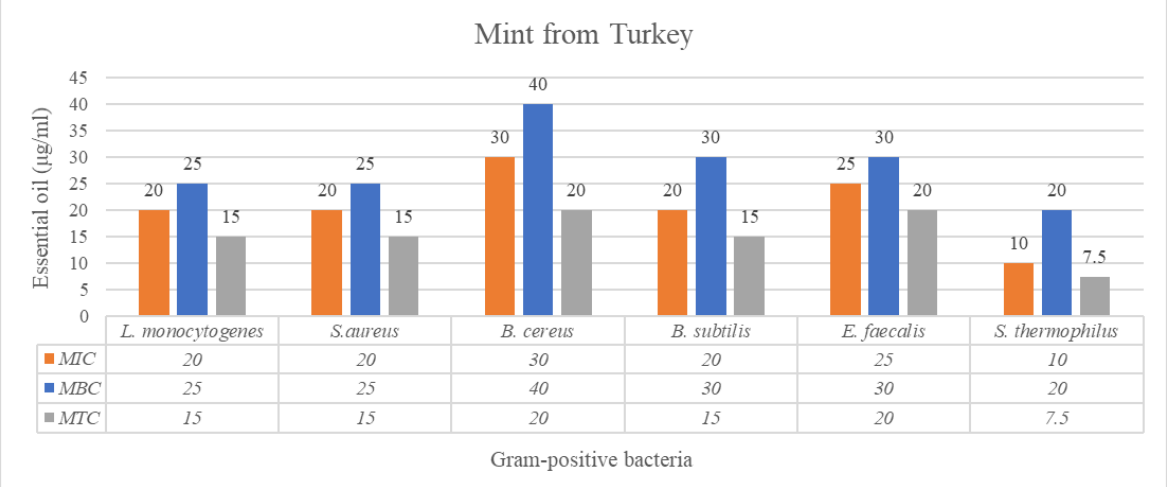

Figure 4.12. Antimicrobial values (MIC, MBC, and MTC) the Turkish mint essential oil against the Gram-positive bacteria

4.1.1.7. Antimicrobial effect of *Matricaria chamomilla* (chamomile) essential oil on Gram-negative bacteria

The antimicrobial values of MIC, MBC, and MTC of the Syrian and Turkish chamomile against Gram-negative bacteria are given in Figures 4.13 and 4.14 respectively. The essential oil MIC values obtained from Syrian chamomile were ranged between 2.5 µg/ml against *S. dysenteriae* and 125 µg/ml against *K. pneumoniae*. MIC values for essential oils that obtained from Turkish chamomile were at the lowest level; 2.5 µg/ml against *S. dysenteriae*, and highest level; 100µg/ml on *K. pneumoniae* so, the Syrian and Turkish chamomile have the same impact against *S. dysenteriae* and *Y. enterocolitica* but the Turkish chamomile essential oil has more impact than Syrian against the rest of studied Gram-negative bacteria with low values of MIC. The MBC values for essential oils obtained from Syrian chamomile ranged between 5 µg/ml against *S. dysenteriae* and 130 µg/ml against *K. pneumoniae*. But the MBC values for essential oils that obtained from Turkish chamomile that ranged between 2.5 µg/ml against *S. dysenteriae* and 125 µg/ml against *K. pneumoniae* so, the Turkish and Syrian chamomile have the same impact against *P. aeruginosa*, but the Turkish chamomile has more impact than Syrian against the rest of studied bacteria with low values of MBC. The MTC values for essential oils obtained from Syrian chamomile were at the lowest level; 1.25 µg/ml against *S. dysenteriae* and the highest level; 90 µg/ml against *K. pneumoniae* comparing with the MTC values for essential oils that obtained from Turkish chamomile were at the lowest level; 1.25 µg/ml against *S. dysenteriae* and the highest level; 80 µg/ml on *K. pneumoniae* so, the Syrian and Turkish chamomile have the same impact against the *S. dysenteriae* and *Y. enterocolitica* but the Turkish essential oil is better than Syrian one against other Gram-negative bacteria with low values for MTC.

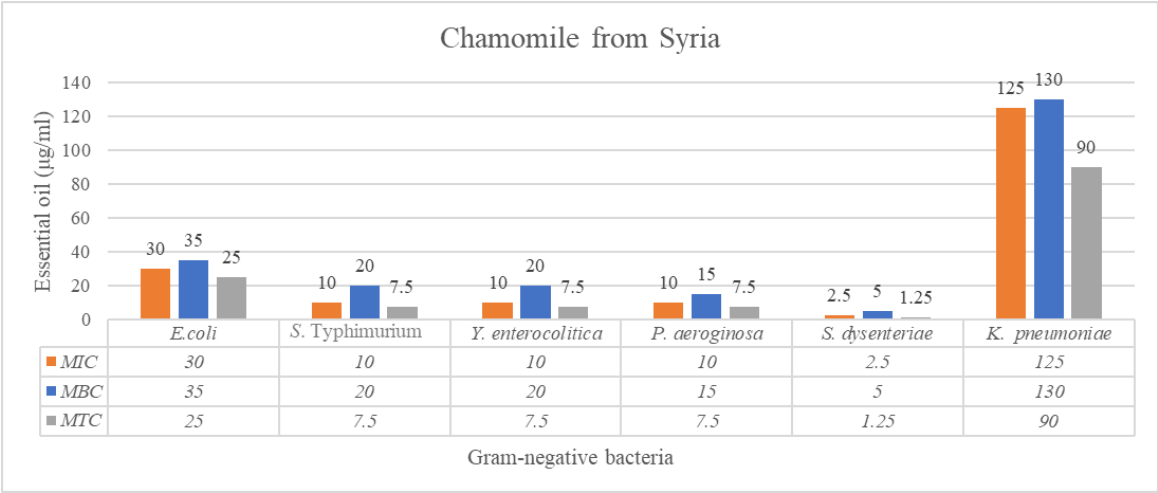

Figure 4.13. Antimicrobial values (MIC, MBC, and MTC) of the Syrian chamomile essential oil against the Gram-negative bacteria

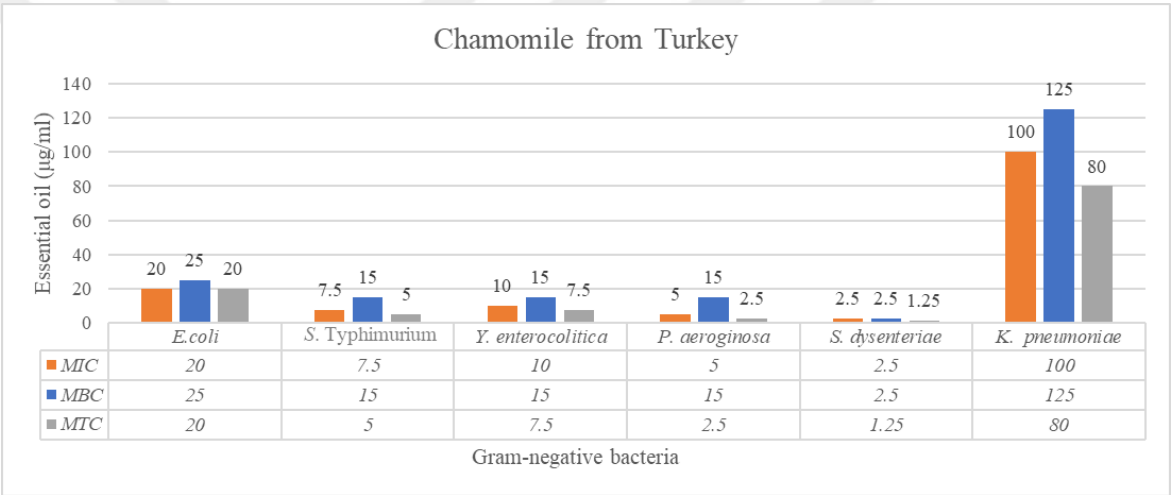

Figure 4.14. Antimicrobial values (MIC, MBC, and MTC) of the Turkish chamomile essential oil against the Gram-negative bacteria

4.1.1.8. Antimicrobial effect of *Matricaria chamomilla* (chamomile) essential oil on Gram-positive bacteria

The antimicrobial values MIC, MBC, and MTC of the essential oils that obtains from Syrian and Turkish chamomile against the Gram-positive bacteria are shown in Figures 4.15 and 4.16 respectively. The essential oil MIC values obtained from Syrian chamomile were at the lowest level; 5 µg/ml against *L. monocytogenes* and the highest level; 25 µg/ml against *B. cereus* and Turkish chamomile was at the lowest level; 10 µg/ml against *S. thermophilus* and *L. monocytogenes* and the highest level; 15 µg/ml against *B. cereus*, *B. subtilis* and *S. aureus* so, the Syrian and Turkish chamomile have the same impact against *L. monocytogenes* but the Turkish ones

have more impact than Syrian ones against the rest of studied bacteria with low values of MIC. The MBC values for essential oils obtained from Syrian chamomile were at the lowest level; 10 µg/ml against *S. thermophilus* and *L. monocytogenes* but the highest level; 30 µg/ml on *B. cereus* comparing with the MBC values for essential oils that obtained from Turkish chamomile were at the lowest level; 7.5 µg/ml on *S. thermophilus* and *L. monocytogenes* but the highest level; 30 µg/ml against the rest ones. They have the same impact against the *E. faecalis*, but the Turkish chamomile essential oil has more impact than Syrian against the rest with low MBC values. The MTC values for essential oils obtained from Turkish chamomile were at the lowest level; 2.25 µg/ml against *L. monocytogenes* and the highest level; 10 µg/ml on against *B. cereus*, *B. subtilis*, and *S. aureus*, has more impact against the studied Gram-positive bacteria with low values of MTC comparing with MTC values for essential oils that obtained from Syrian chamomile were at the lowest level; 2.5 µg/ml against *L. monocytogenes* the highest level; 20 µg/ml against *B. cereus*.

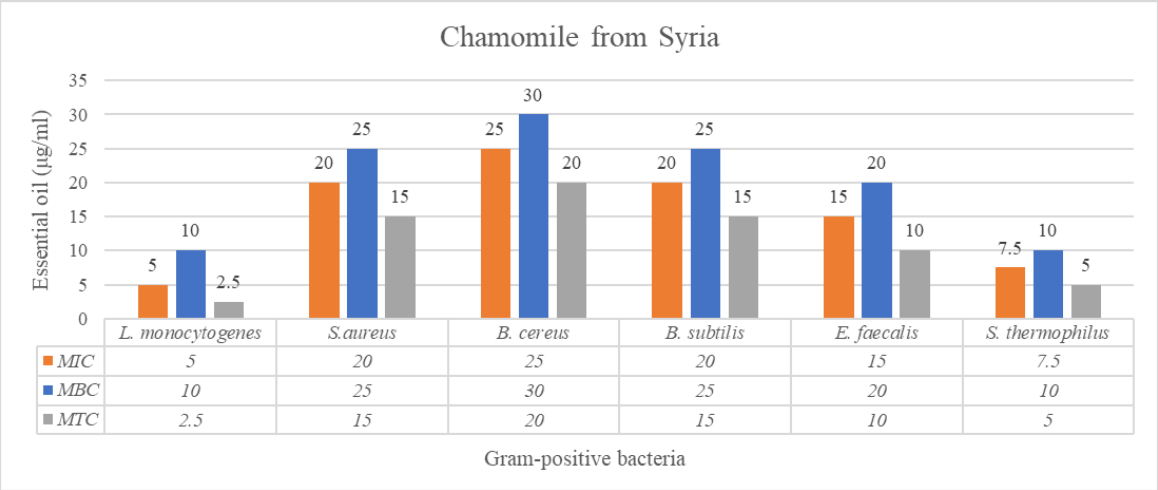

Figure 4.15. Antimicrobial values (MIC, MBC and MTC) of the Syrian chamomile essential oil against the Gram-positive bacteria

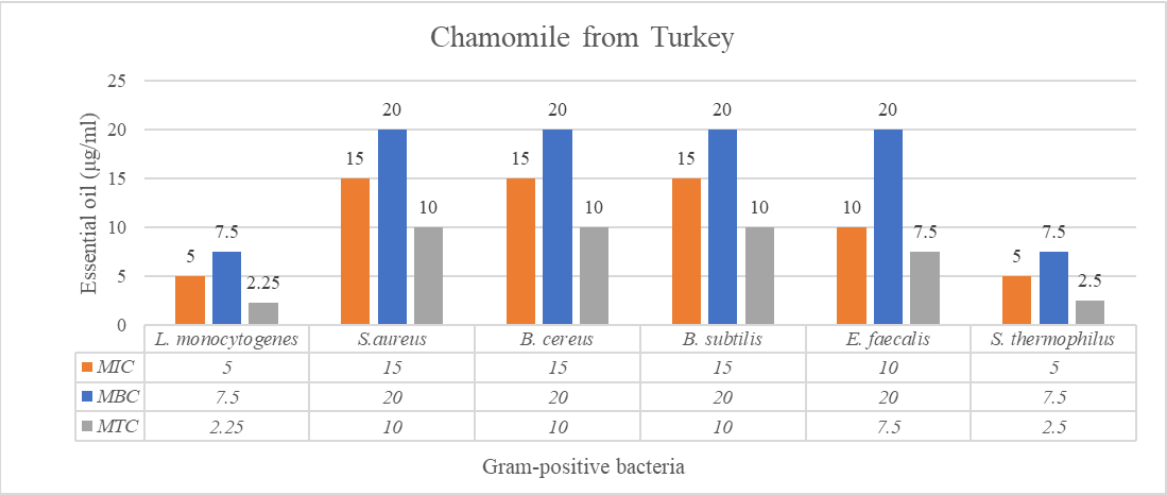

Figure 4.16. Antimicrobial values (MIC, MBC, and MTC) of the Turkish chamomile essential oil against the Gram-positive bacteria

4.1.1.9. Antimicrobial effect of *Ocimum basilicum* (basil) essential oil on Gram-negative bacteria

The antimicrobial values; MIC, MBC and MTC of the Syrian and Turkish basil against the Gram-negative bacteria are given in the Figures 4.17 and 4.18 respectively. The essential oil MIC values obtained from Syrian basil were ranged between 20 µg/ml against *E. coli*, and 60 µg/ml against *K. pneumoniae* which are higher than the MIC values for essential oils that obtained from Turkish basil were ranged between 7.5 µg/ml on *E. coli* and 50 µg/ml on *K. pneumoniae*. That means the Turkish essential oil has higher antimicrobial effects with low concentration against Gram-negative than Syrian ones. These results are not agreed with findings done by (Fei Lv et al., 2011) against *E. coli* that ranged between 0.15 and 5 µg/ml. The justification of this high effect of basil essential oils because the existence of a significant amount of caryophyllene, α-caryophyllene, carvacrol, eugenol, D-limonene and 2-pentanoylfuran, respectively, in addition that the number of components in relatively low concentrations, such as Eugenol, caryophyllene, α-caryophyllene, could also be expected to make a significant contribution to the antimicrobial activity. The MBC values for essential oils that obtained from Syrian basil ranged between 30 µg/ml on *E. coli* and 75 µg/ml on *K. pneumoniae* which are higher than MBC values for essential oils that obtained from Turkish basil that ranged between 12.5 µg/ml on *E. coli* and 60 µg/ml on *K. pneumoniae*. So, the Turkish essential oil has a better impact against these Gram-negative bacteria with

low values of MBC. The MTC values for essential oils that obtained from Syrian basil were ranged between 15 µg/ml against *E. coli* and 55 µg/ml against *K. pneumoniae* and Turkish basil essential oil were ranged between 5 µg/ml on *E. coli* and 50 µg/ml against *P. aeruginosa*, so the Turkish and Syrian have the same impact against the *S. dysenteriae* and the Syrian essential oil has more impact than Turkish against *P. aeruginosa* but the Turkish ones have more impact than Syrian against the rest of studied Gram-negative bacteria with low values of MTC.

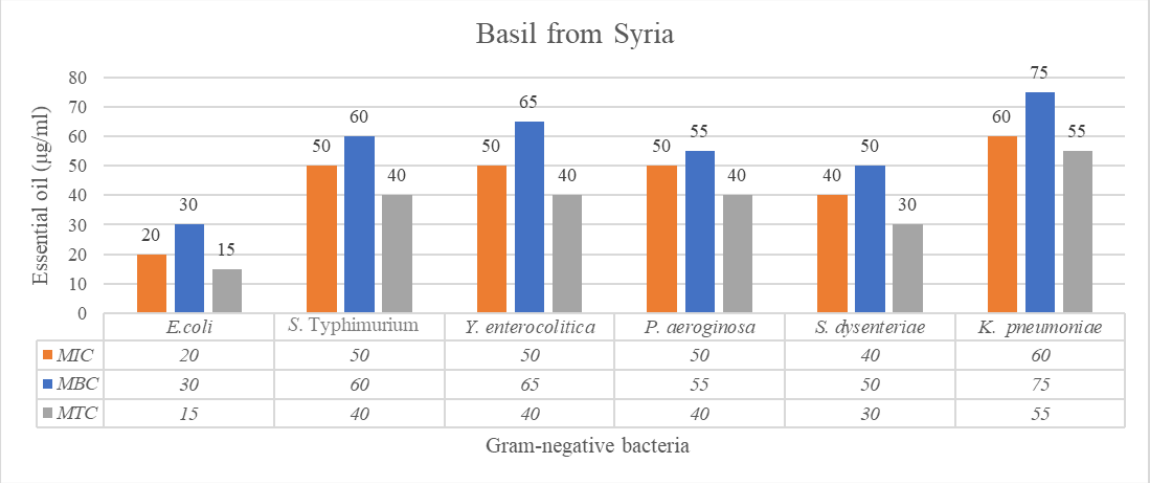

Figure 4.17. Antimicrobial values (MIC, MBC, and MTC) of the Syrian Basil essential oil aginst the Gram-negative bacteria

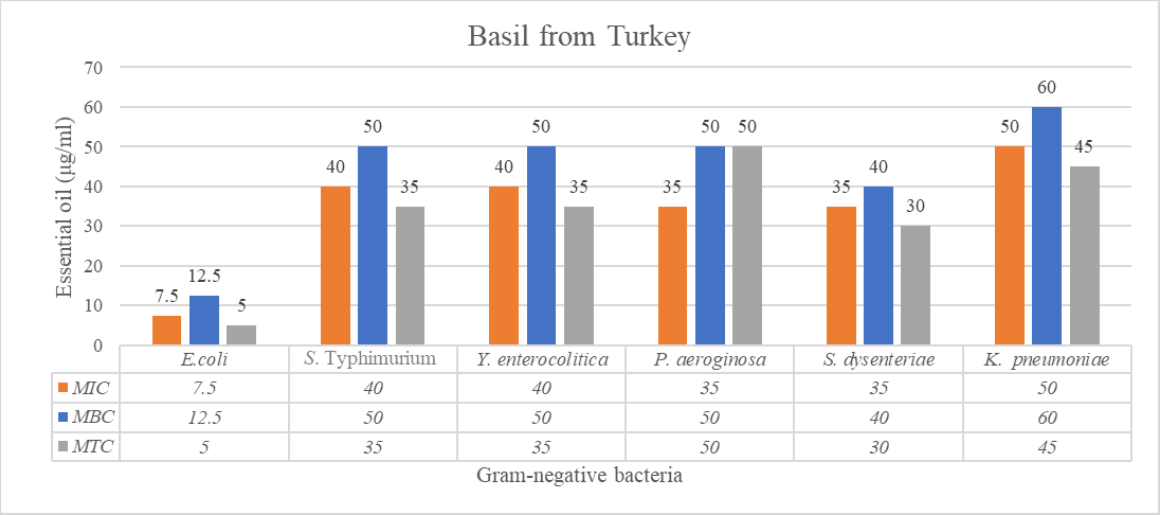

Figure 4.18. Antimicrobial values (MIC, MBC, and MTC) of the Turkish basil essential oil aginst the Gram-negative bacteria

4.1.1.10. Antimicrobial effect of *Ocimum basilicum* (basil) essential oil on Gram-positive bacteria

The antimicrobial values MIC, MBC, and MTC of the Syrian and Turkish basil against the gram-positive bacteria are given in Figures 4.19 and 4.20 respectively. The essential oil MIC values obtained from Syrian basil were between 10 µg/ml against *S. thermophilus* and 60 µg/ml against *B. cereus*, which were close to the MIC values for essential oils that obtained from Turkish basil ranged between 7.5 µg/ml against *S. thermophilus* and 60 µg/ml against *B. cereus*. They have the same impact against *B. cereus* and *B. subtilis* but against the other studied Gram-positive; the Turkish MIC values are lower than Syrian ones; that means the Turkish basil has more impact than Syrian ones so, the Turkish basil is better to be used against them. Compared to other studies that done by (Lv et al., 2011) the MIC of basil essential oils was 1.25 µg/ml for the *S. aureus* and 0.625 µg/ml against *B. subtilis* results are much lower than our results. The MBC values for Syrian basil ranged between 15 µg/ml against *S. thermophilus* and 70 µg/ml against *B. cereus*. However, for Turkish basil that ranged between 10 µg/ml against *S. thermophilus* and 65 µg/ml against *B. cereus* so, the Syrian essential oil has more impact than Turkish against *S. aureus* with low MBC values, but the Turkish ones have more impact against the rest of studied Gram-positive bacteria compared with the Syrian ones. MTC values for essential oils obtained from Syrian basil were at the lowest level; 7.5 µg/ml on S. *thermophilus*, and the highest level; 55 µg/ml on *B. cereus* but the higher MTC value comparing with the MTC values for essential oils that obtained from Turkish basil that ranged between 5 µg/ml against *S. thermophilus* and 55 µg/ml against *B. cereus*. So, they have the same impact against *B. cereus*, but Turkish basil is better to be used against the rest of studied Gram-positive bacteria than Syria because Turkish ones have fewer MTC values than Syrian.

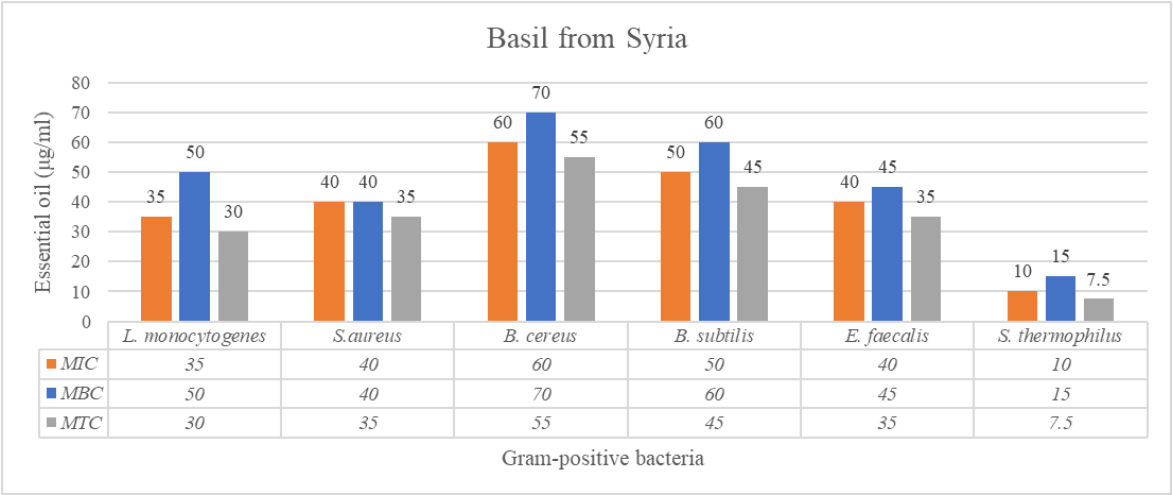

Figure 4.19. Antimicrobial values (MIC, MBC, and MTC) of the Syrian Basil essential oil against the Gram-positive bacteria

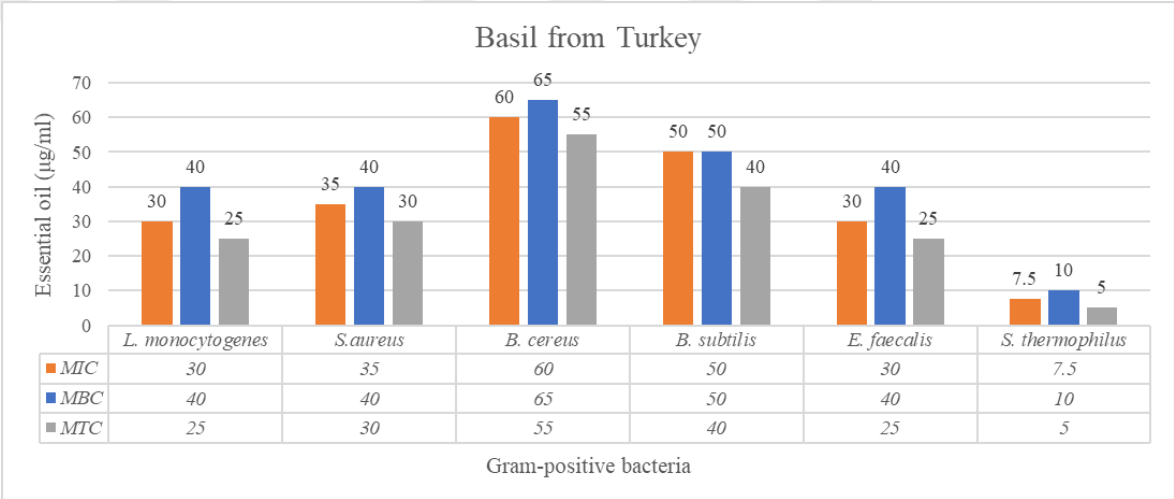

Figure 4.20. Antimicrobial values (MIC, MBC, and MTC) of the Turkish basil essential oil against the Gram-positive bacteria

4.1.2. Antimicrobial effect of plant essential oils from Turkey and Syria on molds and yeasts

In this thesis study; MIC, MFC and MTC values of plant essential oils obtained from Syria and Turkey are measured against yeasts and molds besides bacteria.

4.1.2.1. Antimicrobial effect of *Thymbra spicata* (thyme) essential oil on molds and yeasts

The antimicrobial values of MIC, MFC, and MTC of the Turkish and Syrian thyme against the molds and yeasts are given in Figures 4.21 and 4.22 respectively. The

essential oil MIC values from Syrian thyme were ranged between 2.5 µg/ml on *A. ochraceus* and *A. flavus* and 5 µg/ml on the rest of the fungi. Compared with the MIC values for Turkish thyme which were between 1.25 µg/ml on *A. ochraceus*, *P. expansum* and *A. flavus*, and 5 µg/ml on *F. graminearum*. So, the Syrian and Turkish thyme essential oil has the same impact against *F. graminearum*, but the Turkish ones have more impact against the rest of the studied fungi with a low value of MIC. These results against *A. ochraceus* are met with the results that found by (Giweli et at., 2011) where the values of MIC were ranged from0.005 to 1.5 µg/ml and agreed with Turkish thyme but not with Syrian essential oil. But their results did not meet with the Turkish and Syrian essential oil against *A. niger* where the values of MIC were ranged from 0.02 to 1.5 µg/ml and agreed with the findings of (Markovic et al., 2011) where their MIC values against *A. niger* and *A. ochraceus* where their findings were between 0.05 and 5 µg/ml and between 0.05 and 25 µg/ml respectively. MFC values for essential oils that obtained from Syrian thyme were ranged between 5 µg/ml against *C. rugosa* and 10 µg/ml against the other molds except for *A. flavus* and *S. cerevisiae* which is 7.5 µg/ml. In general, these results were close to Turkish thyme results that ranged between 2.5 µg/ml against *A. ochraceus* and 10 µg/ml against *R. oryzae*. Syrian and Turkish essential oil thyme have the same impact against *R. oryzae*and *C. rugosa,*but the other Turkish ones have more impact against the other fungi with low values of MFC. The MFC results for the essential oils from the Syrian and Turkish against fungi did not match with the results of (Giweli et al., 2011) where the results that they found against of these fungi were between 0.01 and 2 µg/ml. These results are agreed with the findings of (Markovic et al., 2011) where their MFC values against *A. niger* and *A. ochraceus* were between0.05 µg/ml to 25 µg/ml. On the other hand, MTC values for essential oils obtained from Syrian thyme was at the lowest level; 1.25 µg/ml on *S. cerevisiae* and *C. rugosa* and the highest level; 5 µg/ml on *R. oryzae* that did not be aligned with the Turkish results that obtained from Turkish thyme which ranged between 0.5 µg/ml against *A. orchaceus* and 2.5 µg/ml against. *P. expansum*, *R. oryzae*, and *F. graminearum*. Where the Turkish and Syrian essential oil thyme have the same impact against *F. graminearum* and *P. expansum*, but the Turkish essential oil thyme has more impact against the remain fungi with low values of MTC.

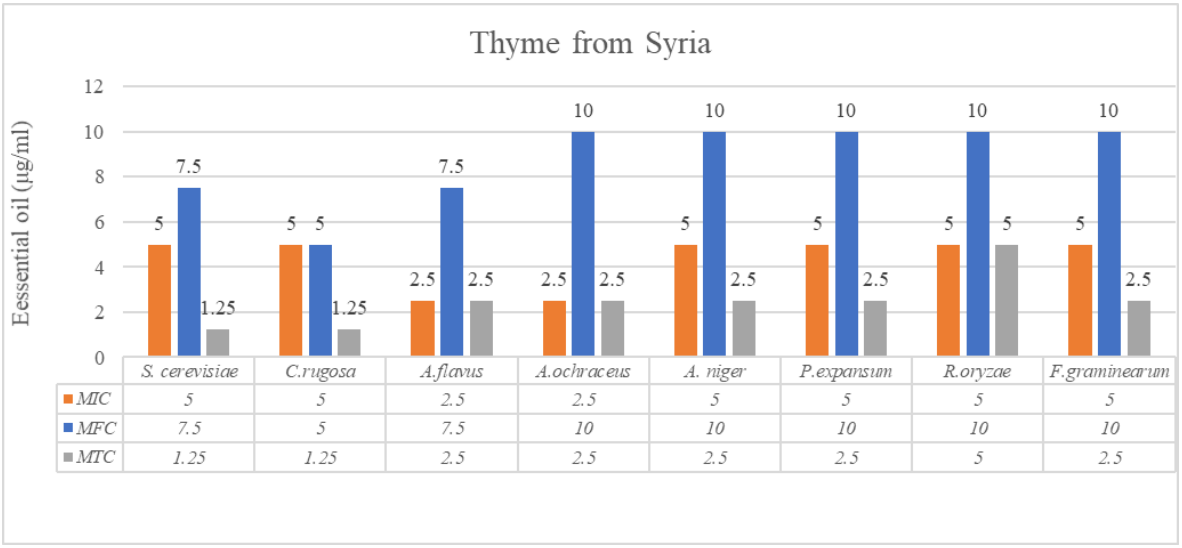

Figure 4.21. Antimicrobial values (MIC, MFC and MTC) of Syrian thyme essential oil against molds and yeasts

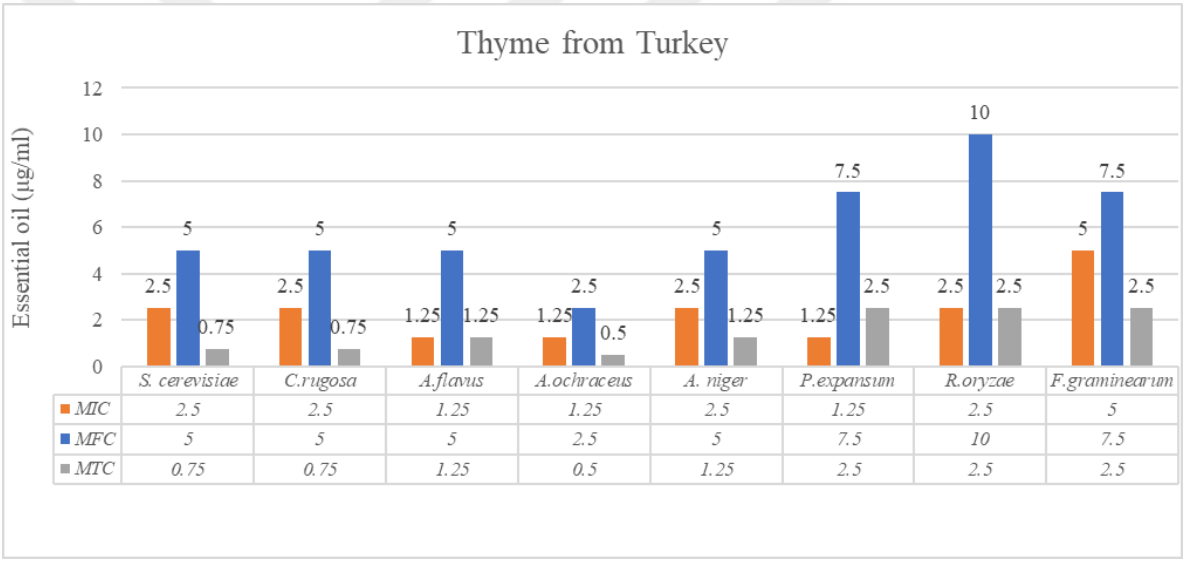

Figure 4.22. Antimicrobial values (MIC, MFC and MTC) of Turkish thyme essential oil against molds and yeasts

4.1.2.2. Antimicrobial effect of *Folium menthae* (mint) essential oil on molds and yeasts

The antimicrobial values of MIC, MFC, and MTC of the Turkish and Syrian mint against the molds and yeasts are given in Figures 4.23. and 4.24. respectively. The essential oil MIC values obtained from Syrian mint were between 5 µg/ml on *A. flavus* and 10 µg/ml on *C. rugosa, A. ochraceus*and *R. oryzae*. Comparing with the MIC values for essential oils that obtained from Turkish mint were the lowest level;

2.5 µg/ml on *A. flavus* and *P. expansum* and the highest level; 10 µg/ml on *F. graminearum*. So, the Syrian mint oil has more impact against *F. graminearum*, and the Turkish mint oil has more impact than Syrian against the rest of fungi with low values of MIC. These results did not agree with the findings that done by (Thosar et at., 2013) where the MIC values were 0.5 ml/ml against yeast *C. albicans*.

MFC values for essential oils obtained from Syrian mint were at the lowest level; 10 µg/ml on *A. ochraceus* and*P. Expansum,* the highest level; 25 µg/ml on *R. oryzae* but the MFC values for essential oils that obtained from Turkish mint were at the lowest level; 5 µg/ml on *A. ochraceus*and*A. niger* and the highest level; 20 µg/ml on *R. oryzae*. So, the Turkish essential oil mint has more impact against all the studied fungi except *P. expansum*and *C. rugosa*where they have the same impact against the two. For the MTC values of essential oils obtained from Syrian mint were between 2.5 µg/ml on *S. cerevisiae* and 15 µg/ml on *R. oryzae*. Comparing with MTC values for essential oils obtained from Turkish mint that ranged between 1.25 µg/ml on *A. flavors* and *S. cerevisiae*, and 10 µg/ml against *F. graminearum* and *R. oryzae*. The Turkish and Syrian essential oil mint have the same impact against *F. graminearum*, and the Syrian mint has more impact against *P. expansum*, but the Turkish essential oil mint has more impact against the remain fungi with low values of MTC.

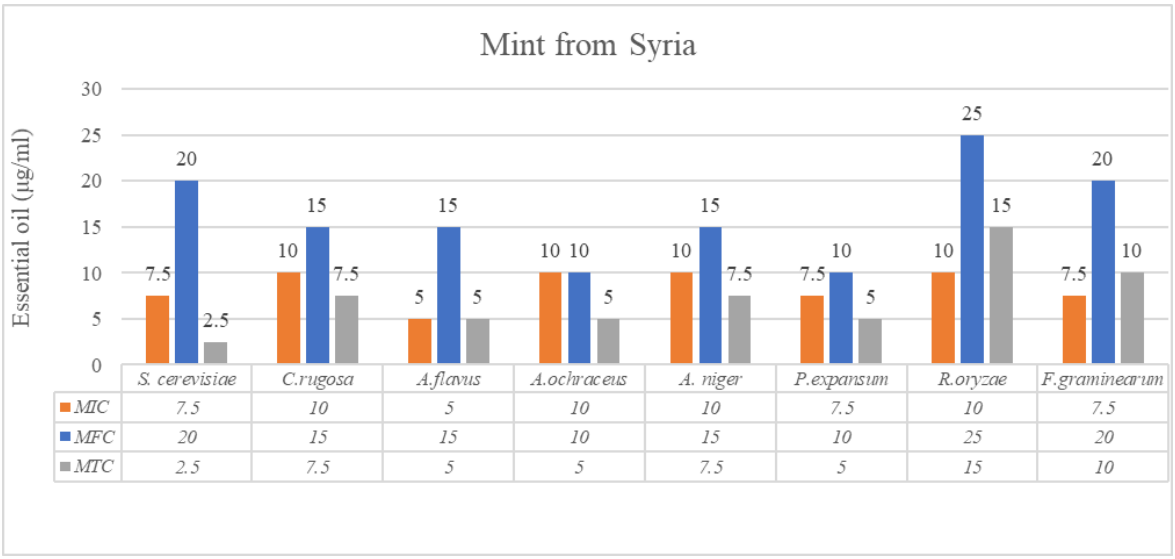

Figure 4.23. Antimicrobial values (MIC, MFC, and MTC) of Syrian mint essential oil aginst molds and yeasts

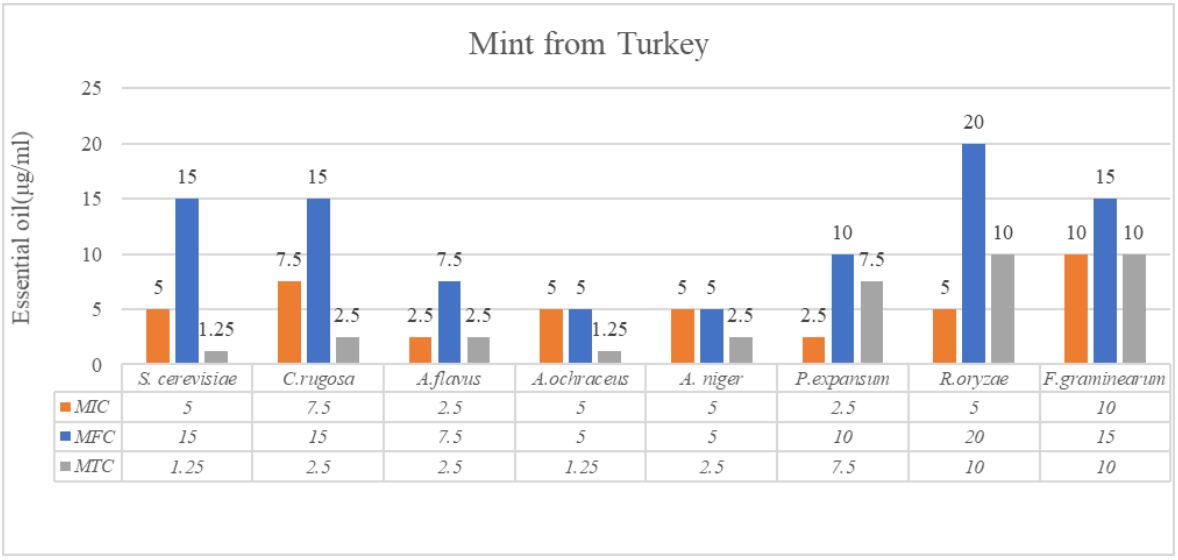

Figure 4.24. Antimicrobial values (MIC, MFC, and MTC) of Turkish mint essential oil on molds and yeasts

4.1.2.3. Antimicrobial effect of *Matricaria chamomilla* (chamomile) essential oil on molds and yeasts

The antimicrobial values MIC, MFC, and MTC of the Turkish and Syrian chamomile against molds and yeasts are given in Figures 4.25. and 4.26. respectively. The essential oil MIC values obtained from Syrian chamomile were ranged between 2.5 µg/ml on *S. cerevisiae* and 7.5 µg/ml on *A. ochraceus*. Comparing with the MIC values for essential oils that obtained from Turkish chamomile were between 1.25 µg/ml on *S. cerevisiae* and *C. rugosa*, and 5 µg/ml on *A. ochraceus* and *A. niger* these results have the same impact against *A. niger*, but essential oils that extracted from the Turkish plants have more impact against the rest of studied fungi with low value of MIC. The MFC values for essential oils that obtained from Syrian chamomile were ranged between the highest level10 µg/ml on *C. rugosa* and *P. expansum*and lowest at7.5µg/ml against the rest. They were close to the MFC values for essential oils obtained from Turkish chamomile were ranged between 5 µg/ml on*S. cerevisiae,A. flavus,* and *F. graminearum*, and 10 µg/ml on *A. niger*, so the Turkish and Syrian essential oil have the same impact against*R. oryaze*and*A. ochraceus,* the Syrian has more impact than Turkish against *A. niger* but the Turkish has more impact than Syrian against the rest. The MTC values for essential oils that obtained from Syrian chamomile were at the lowest level; 1.5 µg/ml on *R. oryaze* and the highest level; 5 µg/ml on *C. rugoza*, *A. niger* and *P. expansum*. And

comparing with the MTC values for essential oils that obtained from Turkish chamomile were at the lowest level; 0.75 µg/ml on *R. oryaze* and *F. graminearum*, and the highest level; 5 µg/ml on *A. niger* and *C. rugosa*. Two essential oils have the same impact against *C. rugosa*, *A. flavus*, and *A. niger*. The Turkish essential oil has more impact against the rest of the studied fungi with low values of MTC.

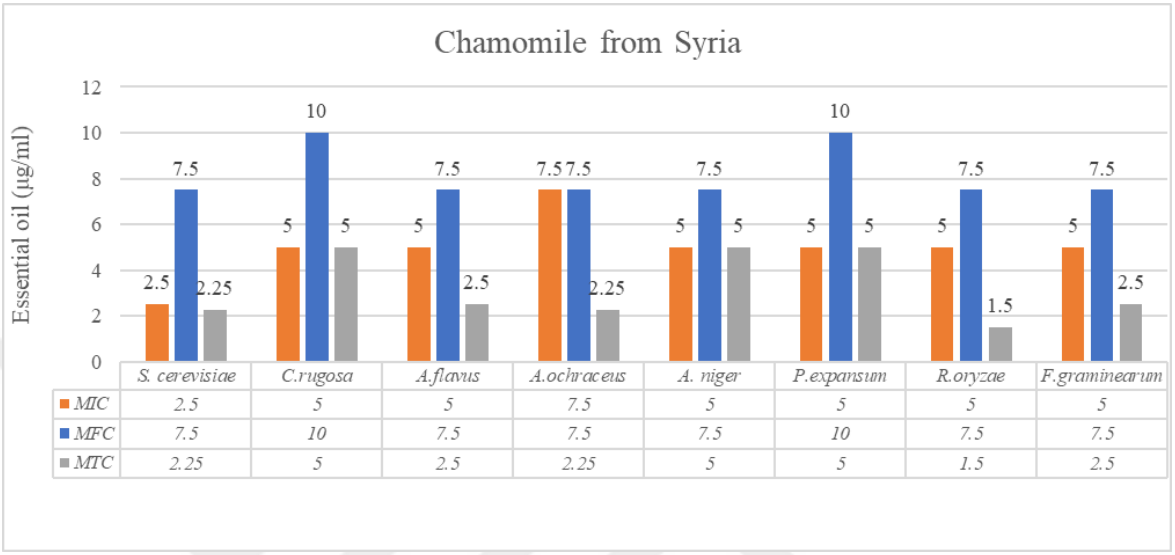

Figure 4.25. Antimicrobial values (MIC, MFC and MTC) of Syrian chamomile essential oil against molds and yeasts

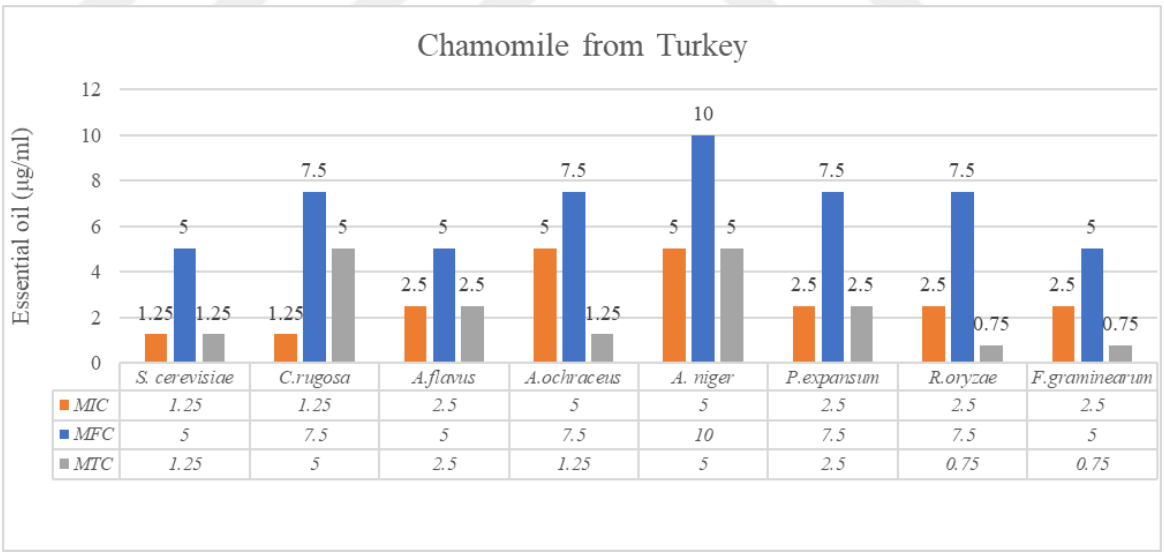

Figure 4.26 Antimicrobial values (MIC, MFC and MTC) Turkish chamomile essential oil against the molds and yeasts

4.1.2.4. Antimicrobial effect of *Ocimum basilicum* (basil) essential oil on molds and yeasts

The antimicrobial values of MIC, MFC, and MTC of the Turkish and Syrian basil against the molds and yeasts are given in Figures 4.27. and 4.28. respectively. The values of MIC of essential oils that obtained from Syrian basil comparing with the values of MIC for essential oils that obtained from Turkish basil were the lowest level; 10 µg/ml on *C. rugosa, A. ochraceus* and *A. flavus* the highest level; 20 µg/ml on the other fungi. The Syrian essential oil has more impact against*C. rugosa* and*A. flavus* than Turkish, while they have the same impact against *A. niger* and Turkish essential oil has more impact than Syrian against the rest of studied fungi with a low value of MIC. Comparing with other studies done by (Fei Lv et al., 2011) the MIC value of essential oil that extracted from basil were ranged from 0.3 to 2.5 µg/ml on *S. cerevisiae*. There is no aligned between the two studies. The MFC values for essential oils that obtained from Syrian basil that ranged between 10 µg/ml on *C. rugosa* and 25 µg/ml on other fungi except *S. cerevisiae* and *F. graninarum*; comparing with the MFC values for essential oils that obtained from Turkish basil that ranged from 10 µg/ml on *C. rugosae* to 20 µg/ml on other fungi (except *S. cerevisiae , A. ochraceus* and *F. graminearum*); so the two essential oils have the same impact against *C. rugosa* but the Turkish essential oil has more impact than Syrian one against the other studied fungi with low values of MFC. The MTC values for essential oils that obtained from Syrian basil were at the lowest level; 5 µg/ml on *S. cerevisiae* and *C. rugosa* and the highest level; 15 µg/ml on other fungi except *F. graminearum*. Comparing with the MTC values for essential oils that obtained from Turkish basil that ranged between 2.5 µg/ml on *C. rugosa* and 15 µg/ml on *A. flavus*; the two essential oils have the same impact against the *A. flavus*, the Syrian basil has has more impact than Turkish against *S. cerevisiae* and the Turkish essential oil has more impact against the rest studied fungi with low values of MTC.

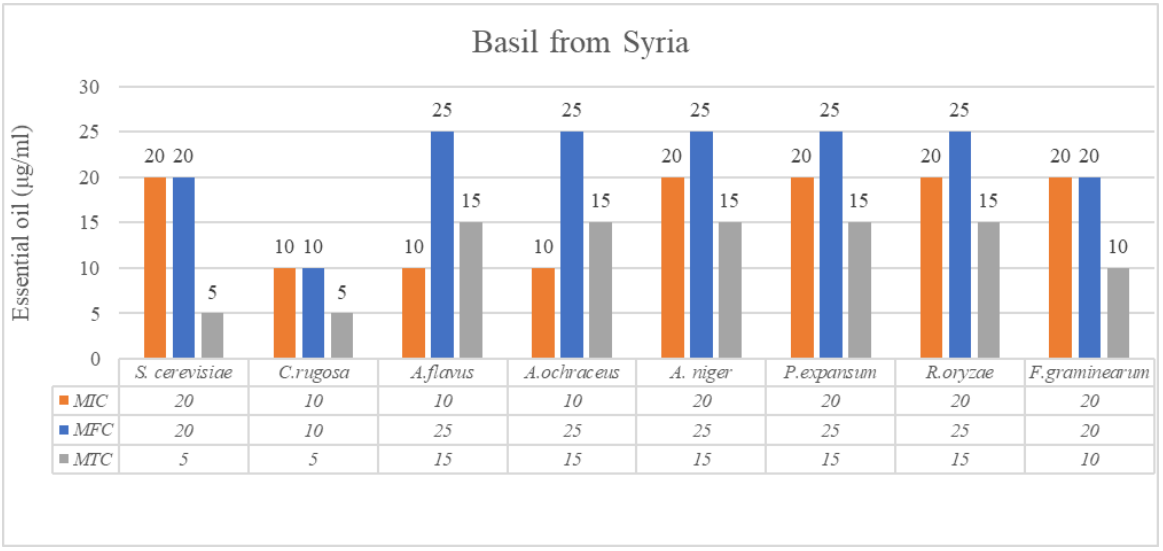

Figure 4.27. Antimicrobial values (MIC, MFC and MTC) of Syrian Basil essential oil against molds and yeasts

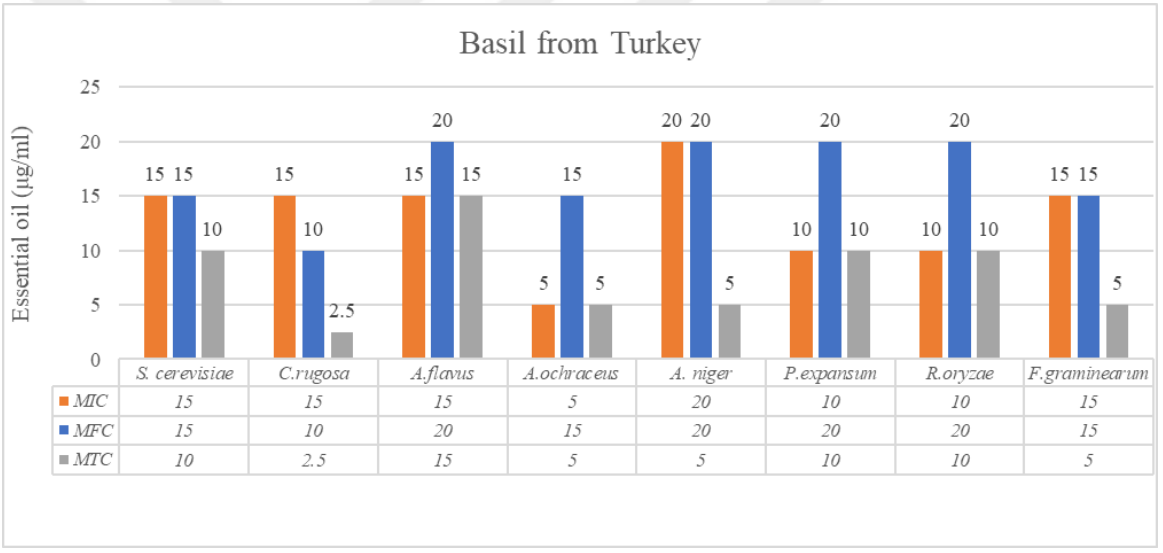

Figure 4.28. Antimicrobial values (MIC, MFC and MTC) of Turkish Basil essential oil against molds and yeasts

4.1.2.5. Antimicrobial effect of *Pimpinella anisum*(anise) essential oil on molds and yeasts

The MIC values for essential oils that obtained from Syrian anise were more than 200 µg/ml and this agree with the MIC values for essential oils that obtained from Turkish anise were more than 200 µg/ml and that belong to the closeness between Syrian and Turkey and the similarity of the climate. As a brief conclusion, both Syrian and Turkish anise essential oils are not inhibitory against molds and yeasts.

4.2. Discussion

MIC, MBC (or MFC) and MTC of essential oils of the Syrian and Turkish plants were compared. These values give information about the minimum concentration of essential oils in a system to be antimicrobial effective against microorganisms. So, the antimicrobial effectiveness of these plant essential oils is determined with these concentrations. Although there are several factors affecting the antimicrobial effects of essential oils. The essential oil's concentration provides information about the usage of these essential oils as antimicrobial agents in a system. Statistical analysis is done on the MIC, MBC and MTC data obtained in this research and essential oils having significantly different effects on microorganisms aimed to be determined. Effect of regional obtained essential oil depending on bacteria classification (Gram-positive and Gram-negative) is analyzed by using One-Way ANOVA and independent sample t-test at the $\alpha=0.05$ level being $p<0.05$ significantly different from the rest of data. Comparison of MIC values according to the regional effect is shown in Table 4.1. All of the MIC values without distinguishing between bacteria type is compared just for two regions Turkey and Syria. Sixty samples and their MIC values are compared with each other by using independent sample t-test. There is no significant difference ($p>0.05$) between Syria and Turkey according to the mean MIC values of essential oil. This means of all MIC values are not significantly different ($p>0.05$) between two regions. There are several differences between groups of bacteria, yeasts, and molds but when we handle all the date together this result shows us Turkey and Syria plants have similar MIC values.

Table 4.1. Mean MIC values (µg/ml) comparison between Syria and Turkey essential oils with the "Independent- samples t-Test" for bacteria

Country	n	Mean	t	p
Syria	60	27.12±2.90	1.661	0.099
Turkey	60	20.84±2.42		

MIC values of essential oils from two different regions are compared with one-way ANOVA test and subsets are analyzed by using Duncan post-hoc test. Table 4.2. shows that thyme from Syria and Turkey are significantly effective ($p<0.05$) with MIC values than other plants meaning that thyme essential oil has a more antimicrobial effect on selected bacteria than other plant essential oils. Anise, mint, chamomile from Syria and anise, mint, basil from Turkey have a no significant

difference from each other (p> 0.05) upon MIC values obtained., There are significant (p<0.05) differences among essential oils antimicrobial effects that have different letters.

The MIC values of chamomile from Syria were significantly different (p< 0.05) than anise and basil essential oils from Syria but not significant (p> 0.05) with mint. Also, Figure 4.29. shows these differences clearly showing that thyme essential oils are significantly more effective than others. Syrian basil has a higher MIC means than all other plants.

Table 4.2. Mean MIC values comparison between essential oils from Turkey and Syria with the "One-Way ANOVA" by "Duncan Post-Hoc Test" for bacteria

Essential oil	**n**	**Mean**	**Essential oil**	**n**	**Mean**
Essential oil from Syria			**Essential oil from Turkey**		
Thyme	12	0.60 ± 0.11^{a}	Thyme	12	0.45 ± 0.09^{a}
Anise	12	37.08 ± 5.20^{de}	Anise	12	28.75 ± 4.46^{bcde}
Mint	12	34.58 ± 6.16^{cde}	Mint	12	$24,580\pm3.96^{bcd}$
Chamomile	12	21.25 ± 7.55^{bc}	Chamomile	12	$15,83\pm6.03^{b}$
Basil	12	42.08 ± 4.33^{e}	Basil	12	34.58 ± 4.40^{bc}

Means for groups in homogeneous subsets are displayed in same letters at 0.05 level (p< 0.05, significant). F =9,8088, p = 0.0001

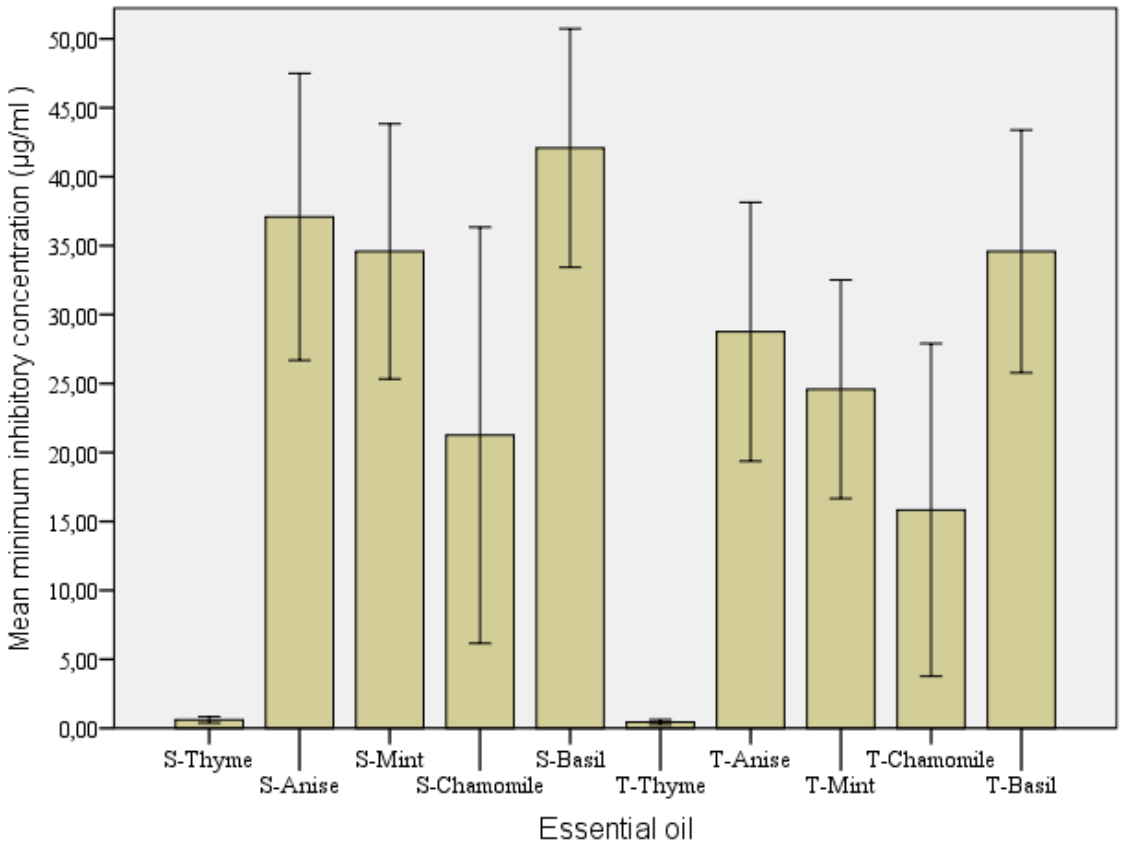

Figure 4.29. Mean MIC values of essential oils from Syria and Turkey for bacteria (S=Syria, T=Turkey)

Another comparison can be done among bacteria type being Gram-positive and Gram-negative bacteria. Means of MIC values of Syrian and Turkish plant essential oils are compared according to effects on Gram-negative and Gram-positive bacteria. Table 4.3. indicates that there are not significant ($p>0.05$) differences between Gram-negative and Gram-positive bacteria according to mean MIC values. But mean plots given in Figure 4.30. shows that Gram-negative bacteria have higher MIC values than Gram-positive bacteria. Turkish plant essential oils have lower MIC means than Syrian group. Statistically, this difference is not meaningful (Table 4.3.), but we can say that Gram-positive bacteria are still more sensitive than Gram-negative bacteria since Gram-positive bacteria include thicker cell wall structure of lipid amounts than Gram-negative ones.

Table 4.3. Statistical differences of MIC values between Gram-negative and Gram-positive bacteria from Syria and Turkey with "One-Way ANOVA" by "Duncan".

Bacterial group	n	Mean	t	p
Essential oil from Syria			1.296	0.279
Gram-negative	30	29.68±4.61[a]		
Gram-positive	30	24.57±3.53[a]		
Essential oil from Turkey				
Gram-negative	30	22.16±3.76[a]		
Gram-positive	30	19.51±3.11[a]		

Means for groups in homogeneous subsets are displayed in same letters at 0.05 level (Duncan), p< 0.05, significant), p = 0.086

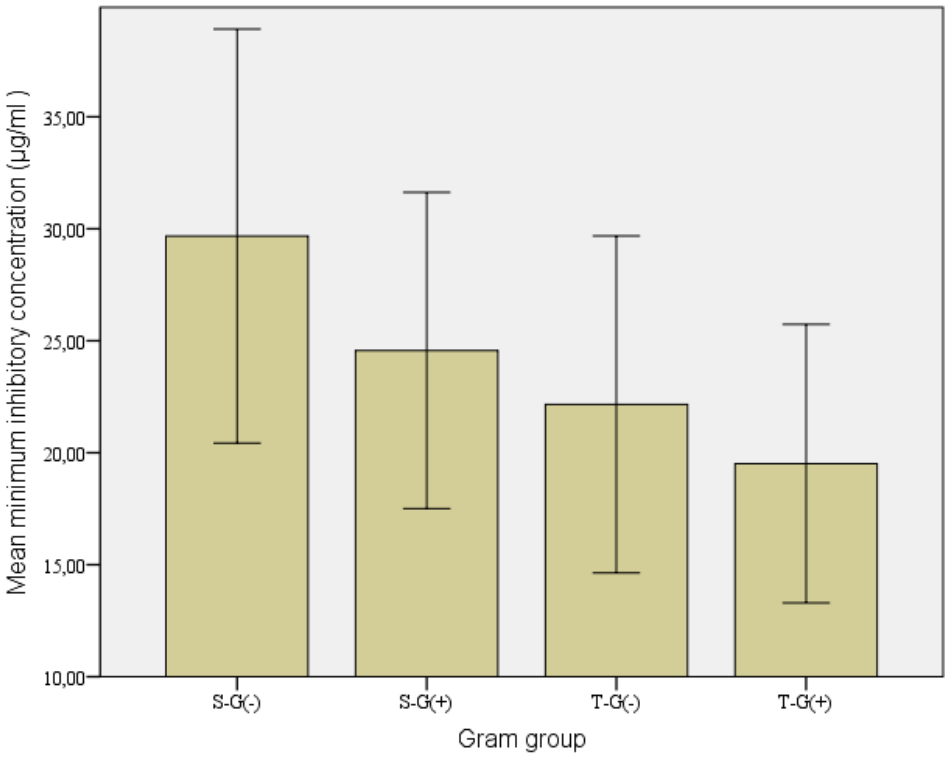

Figure 4.30. Mean MIC values of essential oils from Syria and Turkey for gram groups of bacteria (S=Syria, T=Turkey, G (-) =Gram-negative, G (+) =Gram-posivtive)

For mean MIC values, bacterial effects are shown in Table 4.4. There are significant (p<0.05) effects of mean MIC values of essential oils from Syria and Turkey on *K. pneumoniae* when compared with other bacteria except essential oils from Syria on

B. cereus. There are no significant differences (p> 0.05) among other bacterial species. But differences in values clearly shown in Figure 4.31. *K. pneumoniae* needs a higher amount of mean essential oil from Syria and Turkey.

Table 4.4. Mean MIC values comparison essential oils from Turkey and Syriaagainst bacteria with the "One-Way ANOVA independent samples t-test"

Bacteria	**n**	**Mean**	**Bacteria**	**n**	**Mean**
Essential oil from Syria			**Essential oil from Turkey**		
E. coli	4	19,10±5,01^{abc}	*E. coli*	4	12,55±4,44^{ab}
S. Typhimurium	4	25,05±4,44^{abc}	*S.* Typhimurium	4	18,55±6,93^{ab}
Y. enterocolitica	4	24,05±9,24^{abc}	*Y. enterocolitica*	4	17,02±6,99^{ab}
P. aeroginosa	4	25,05±8,91^{abc}	*P. aeroginosa*	4	17,05±6,79^{ab}
S. dysanteriae	4	24,05±9,98^{abc}	*S. dysanteriae*	4	19,55±8,79^{abc}
K. pneumoniae	4	60,25±16,51^{d}	*K. pneumoniae*	4	48,25±14,96^{cd}
L. monocytogenes	4	23,25±8,58^{abc}	*L. monocytogenes*	4	19,15±7,39^{abc}
S. aureus	4	19,15±6,68^{abc}	*S. aureus*	4	15,05±6,09^{ab}
B. cereus	4	39,20±12,33^{bcd}	*B. cereus*	4	31,10±10,93^{abc}
B. subtilis	4	32,10±9,61^{abc}	*B. subtilis*	4	26,05±9,37^{abc}
E. faecalis	4	22,15±6,70^{abc}	*E. faecalis*	4	18,15±5,49^{ab}
S. thermophilus	4	11,55±3,80^{ab}	*S. thermophilus*	4	7,60±2,43^{a}

Means for groups in homogeneous subsets (non-significant) are displayed in same letters at 0.05 level (Duncan), p< 0.05, significant). F =1,821, p = 0.023

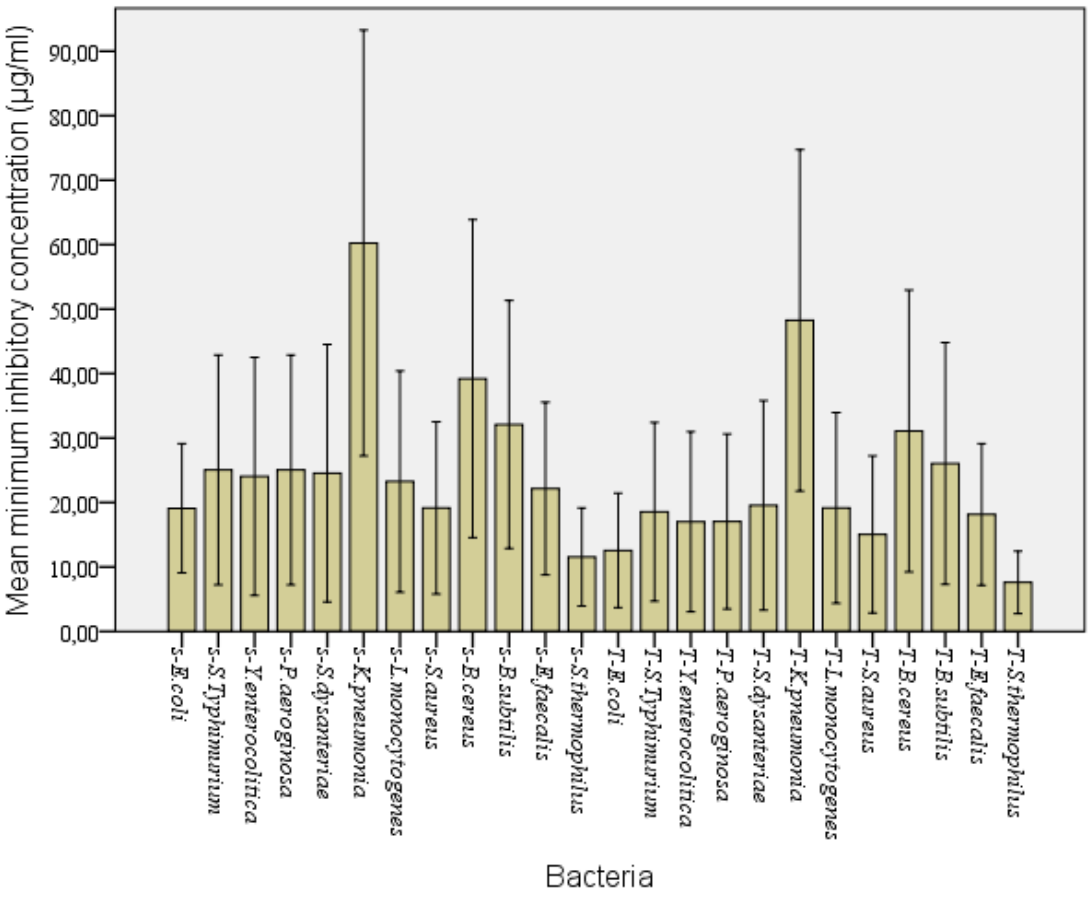

Figure 4.31. Mean MIC values of essential oils of spices from Syria and Turkey against bacteria (1st bacterial group tested with Syrian essential oil)

Comparison of MBC values according to the regional effect is shown in Table 4.5. All of the MBC values without distinguishing between bacteria type is compared just for two regions Turkey and Syria. Sixty samples and their MBC values are compared with each other by using independent sample t-test. There isn't any significant ($p > 0.05$) difference between Syria and Turkey according to MBC values. There are several differences between groups of bacteria, yeasts, and molds but when we handle all the date together this result shows us Turkey and Syria plants have similar MBC values.

Table 4.5. Mean MBC values comparison between Syria and Turkey essential oils with the Independent- Samples t-Test for bacteria

Country	n	Mean	t	p
Syria	60	33.55±3.45	0.910	0.342
Turkey	60	27.39±2.98		

Also, comparisons between essential oils from Syria and Turkey, and their MBC values compared with one-way ANOVA and subsets are determined by using Duncan post-hoc tests. Table 4.6. shows that thyme essential oils have significantly ($p< 0.05$) (lower MBC values) than other essential oils on selected bacteria. Meaning that, thyme essential oils from both Syria and Turkey more effective on bacterial than other plant essential oils at a lower amount of MBC. Other essential oils have similar ($p>0.05$) antimicrobial effect on tested bacteria.

Table 4.6. Mean MBC values comparison between essential oils from Turkey and Syria with the "One-Way ANOVA" and "independent samples t-test" for bacteria

Essential oil	**n**	**Mean**	**Essential oil**	**n**	**Mean**
Spices from Syria			**Spices from Turkey**		
Thyme	12	1.06 ± 0.18^{a}	Thyme	12	0.70 ± 0.11^{a}
Anise	12	45.83 ± 6.51^{cd}	Anise	12	38.33 ± 5.95^{bc}
Mint	12	41.25 ± 4.77^{cd}	Mint	12	33.33 ± 4.94^{bc}
Chamomile	12	$28.33\pm9,14^{bc}$	Chamomile	12	22.29 ± 7.31^{b}
Basil	12	51.25 ± 4.93^{d}	Basil	12	42.29 ± 4.78^{cd}

Means for groups in homogeneous subsets are displayed in same letters at 0.05 level (Duncan); $p< 0.05$, significant). $F = 10,127$, $p = 0.0001$

Mean plot graph in Figure 4.32. shows clearly that thyme essential oils have significantly ($p< 0.05$) (lower MBC values) than all other plant essential oils for bacteria obtained from Syria and Turkey.

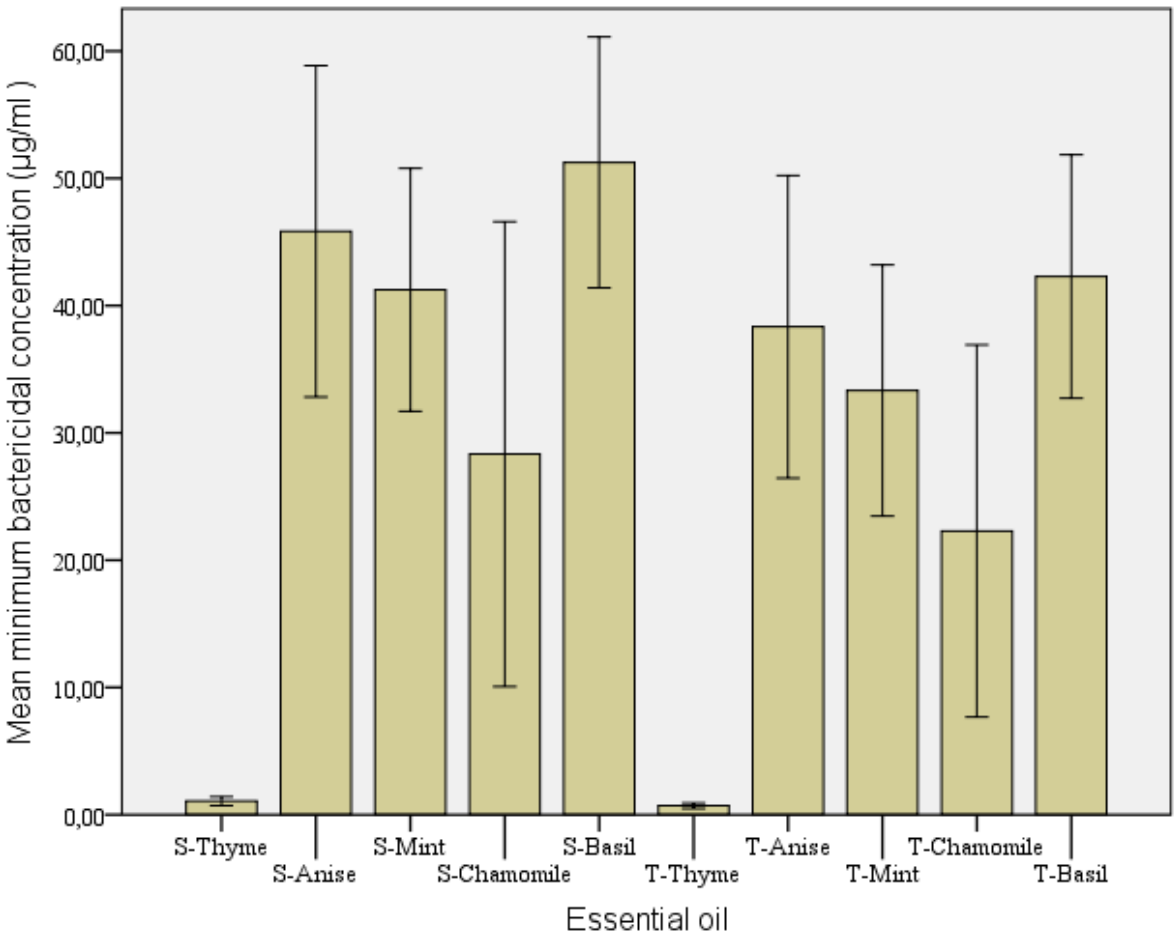

Figure 4.32. Mean MBC values of essential oils from Syria and Turkey for bacteria

For mean MBC values, the bacterial effect is shown in Table 4.7. There are significant ($p<0.05$) effects of mean MBC values of essential oil from Syria and Turkey on *K. pneumoniae* when compared with other bacteria except for essential oil from Turkey on *K. pneumoniae*. There are no significant differences ($p> 0.05$) among other bacterial species. But differences in values clearly shown in Figure 4.33 *S. thermophilus* and *E. faecalis* need a higher amount of mean essential oil from Syria.

Table 4.7. Mean MBC values comparison essential oils from Turkey and Syria against bacteria with the "One-Way ANOVA independent samples t-test"

Bacteria	n	Mean	Bacteria	n	Mean
Essential oil from Syria			**Essential oil from Turkey**		
E. coli	5	24,15±6,06[a]	*E. coli*	5	19,65±5,92[a]
S. Typhimurium	5	32,15±10,08[ab]	*T.* Typhimurium	5	26,10±9,30[a]
Y. enterocolitica	5	29,10±10,93[a]	*Y. enterocolitica*	5	21,05±8,69[a]
P. aeroginosa	5	30,10±9,93[a]	*P. aeroginosa*	5	26,05±8,82[a]
S. dysanteriae	5	31,10±12,02[a]	*S. dysanteriae*	5	23,55±9,90[a]
K. pneumoniae	5	74,35±20,14[c]	*K. pneumoniae*	5	64,30±17,00[bc]
L. monocytogenes	5	30,50±10,14[a]	*L. monocytogenes*	5	24,70±9,31[a]
S. aureus	5	22,20±6,66[a]	*S. aureus*	5	19,10±6,71[a]
B. cereus	5	45,30±14,08[abc]	*B. cereus*	5	38,20±12,56[ab]
B. subtilis	5	40,25±12,35[ab]	*B. subtilis*	5	30,10±9,41[a]
E. faecalis	5	27,25±7,63[a]	*E. faecalis*	5	24,20±6,61[a]
S. thermophilus	5	16,10±5,26[a]	*S. thermophilus*	5	11,65±3,73[a]

Means for groups in homogeneous subsets (non-significant) are displayed in same letters at 0.05 level (Duncan), $p < 0.05$. (significant). $F = 1,875$, $p = 0.018$

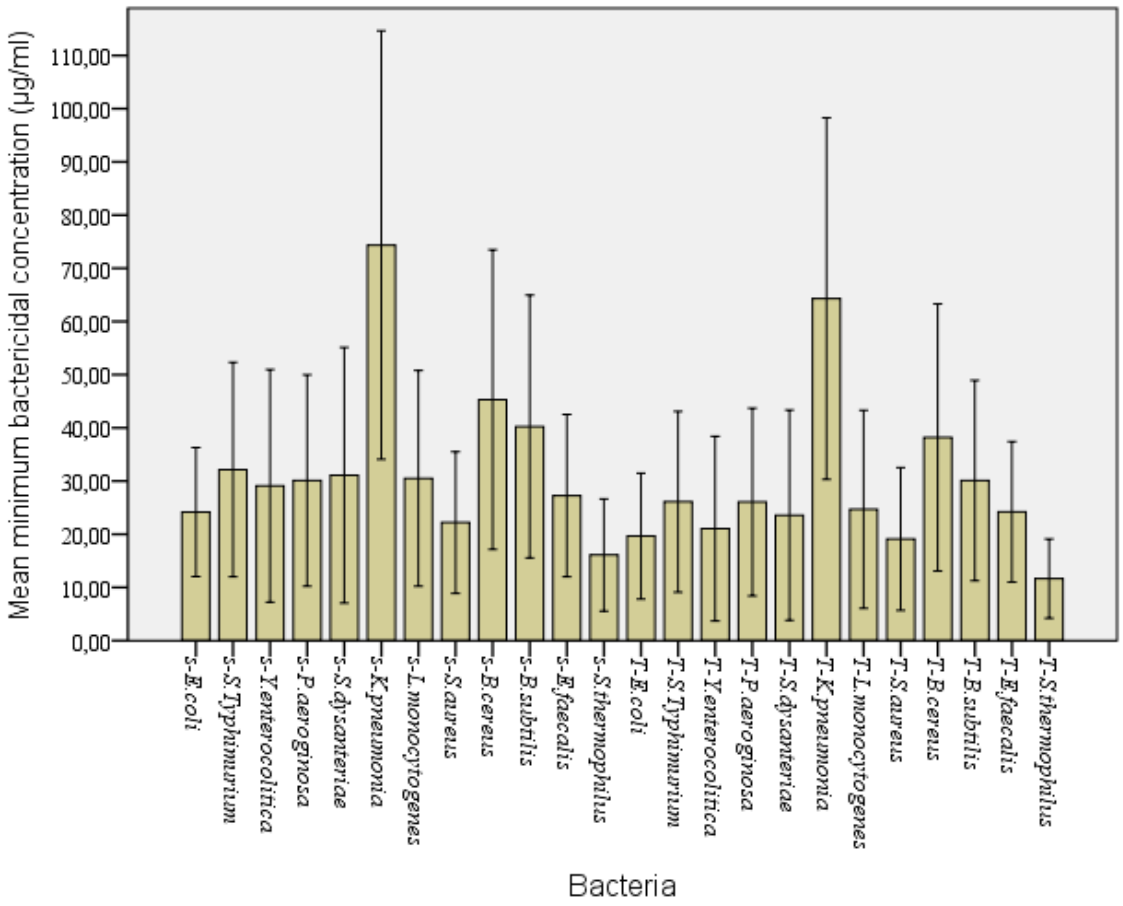

Figure 4.33. Mean MBC values of essential oil from Syria and Turkey against bacteria

Similar results were also obtained for MTC values obtained from Syria and Turkey (Table 4.8.) since it is not significant ($p>0.05$) difference of essential oils between Syria and Turkey. Comparison of essential oils from Syria and Turkey is given in Table 4.9. that shows one-way ANOVA results. As seen, thyme essential oils have significantly lower MTC values ($p< 0.05$) being more effective than all other essential oils.

Table 4.8. Mean MTC values comparison between Syria and Turkey essential oils with the "Independent- Samples t-Test" for bacteria

City	n	Mean	t	p
Syria	60	22.89±2.67	0,656	0.169
Turkey	60	17.94±2.37		

Table 4.9. MTC values comparison between essential oils from Turkey and Syria with the "One-Way ANOVA" and "independent samples t-test" for bacteria

Essential oil	n	Mean		Essential oil	n	Mean
Spices from Syria				**Spices from Turkey**		
Thyme	12	0.40±0.10[a]		Thyme	12	0.26±0.81[a]
Anise	12	30.42±4.46[cd]		Anise	12	24.79±4.56[bcd]
Mint	12	30.83±5.77[cd]		Mint	12	19.79±4.27[bc]
Chamomile	12	17.19±6.93[bc]		Chamomile	12	13.21±6.25[ab]
Basil	12	35.63±6.93[d]		Basil	12	31.67±4.49[cd]

Means for groups in homogeneous subsets are displayed in same letters at 0.05 level (Duncan) $p < 0.05$. (significant). $F = 7.489$, $p = 0.0001$

Mean plots given in Figure 4.34. clearly shows that thyme MTC values are significantly ($p < 0.05$) lower than all other essential oils.

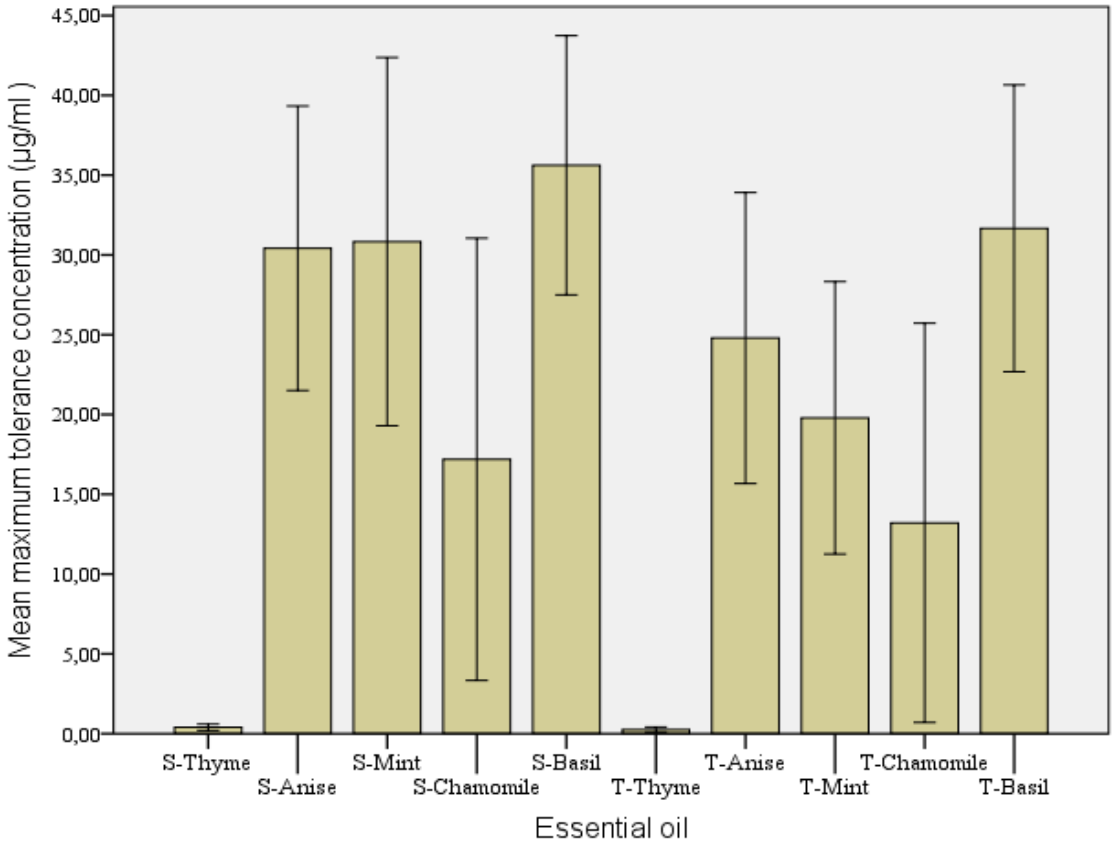

Figure 4.34. MTC mean values of essential oils from Syria and Turkey for bacteria

As a conclusion, it can be said that thyme essential oils regardless of the collected region has an impact on both Gram-negative and Gram-positive bacteria. Turkish and Syrian species show some differences at the micro level but generally, they have similar results according to antimicrobial effects on selected bacteria. *K. pneumoniae* is most resistant bacteria having significantly higher MIC, MBC and MTC values than other bacteria. MIC, MBC and MTC values show similar trends having increased and decreased values in parallel so, having the same antimicrobial because the have the smae components; being neighbor cities Gaziantep (Turkey), Aleppo and Idleb (Syria) plants have similar essential oil compounds and show similar antimicrobial effects. Only basil and chamomile from Turkey showed significantly different ($p<0.05$) effect on selected bacteria due to different climates of regions losing effective essential oils during seasons.

Comparison of MIC values for fungi according to regional effect is shown in Table 4.10. There isn't any significant ($p>0.05$) difference between Syria and Turkey according to MIC values of essential oils. There are several differences between fungi species but when we handle all the date together this result shows us Turkey and Syria plants have similar MIC values.

Table 4.10. Mean MIC values (µg/ml) comparison between Syria and Turkey essential oils with the "Independent- Samples t-Test" for fungi

Country	n	Mean	t	p
Syria	32	9,55±1,05	1,926	0,059
Turkey	32	6,76±0,92		

MIC values of essential oils from two different regions are compared with one-way ANOVA test and subsets are analyzed by using Duncan post-hoc test. Table 4.11 shows that thyme from Turkey and basil from Syria and Turkey are significantly ($p<0.05$) lower than other essential oils meaning that their essential oil has a more antimicrobial effect on selected fungi than other essential oils. Figure 4.35. shows these differences clearly showing that thyme essential oil from Turkey is significantly more effective than others and Syrian basil having higher mean MIC values than all others.

Table 4.11. MIC values comparison between essential oils from Turkey and Syria with the "One-Way ANOVA" for fungi

Essential oil	n	Mean	Essential oil	n	Mean
Essential oil from Syria			**Essential oil from Turkey**		
Thym	6	4,69±0,56^{ab}	Thyme	6	2,96±0,62^{a}
Mint	6	10,62±1,68^{cd}	Mint	6	7,50±1,83 ^{bc}
Chamomile	6	5,62±0,62^{ab}	Chamomile	6	4,06±0,91^{ab}
Basil	6	16,88±1,62^{d}	Basil	6	12,50±1,64^{d}

Means for groups in homogeneous subsets are displayed in same letters at 0.05 level (Duncan). $p < 0.05$, significant. $F = 13{,}926$, $p = 0.0001$

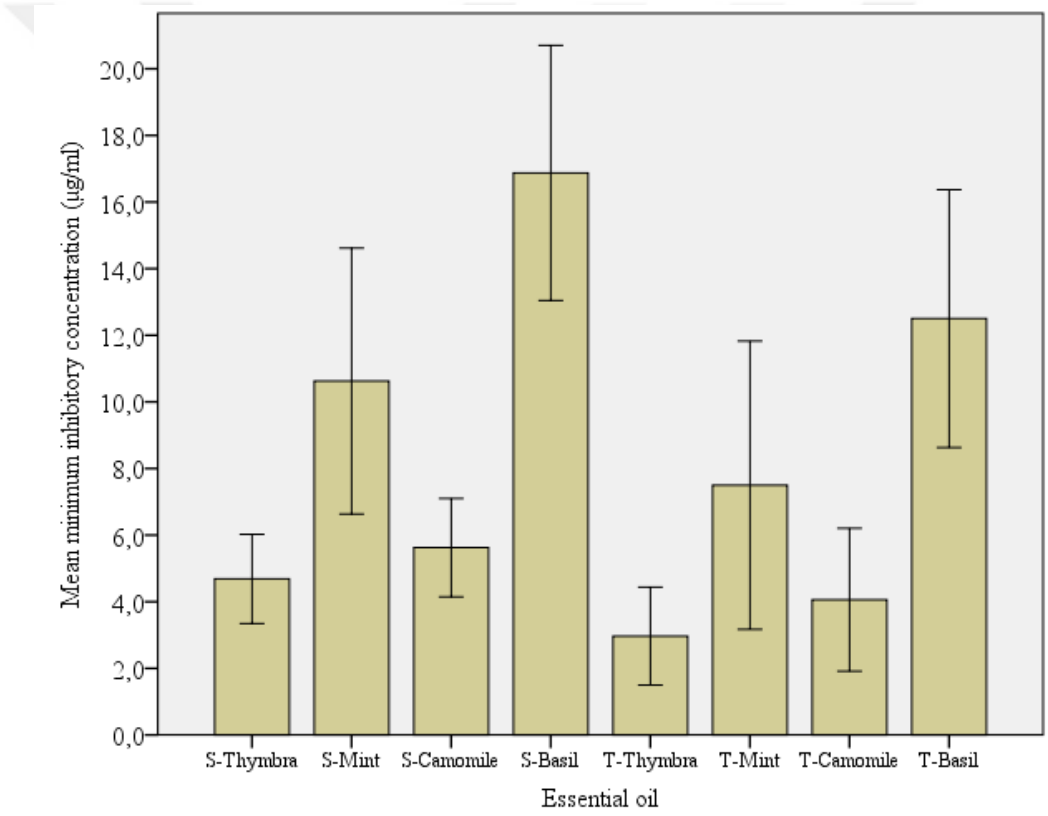

Figure 4.35. Mean MIC mean values of essential oils from Syria and Turkey for fungi

The values of MIC from essential oils that extracted from two different regions are compared with one-way ANOVA test against fungi species and subsets are analyzed by using Duncan post-hoc test. Table 4.12. shows that they have no significant differences ($p>0.05$) among fungi species with respect to mean values of all essential oils from Syria and Turkey. But differences in values clearly shown in Figure 4.36.

S. cerevisiae needs a higher amount of mean essential oil from Syria and *P. expansum* needsa lower amount of mean essential oils from Turkey than others.

Table 4.12. Mean MIC values comparison essential oils from Turkey and Syria against fungi with the "One-Way ANOVA independent samples t-test"

	Essential oil from Syria				**Essential oil from Turkey**		
	Molds	**n**	**Mean**		**Molds**	**n**	**Mean**
1	*A. flavus*	4	5,62±1,57[a]	1	*A. flavus*	4	5,31±3,24[a]
2	*A. ochraceus*	4	6,752±1,77[a]	2	*A. ochraceus*	4	4,69±2,58[a]
3	*A. niger*	4	10,00±3,54[a]	3	*A. niger*	4	8,12±4,00[a]
4	*P. expansum*	4	9,38±3,59[a]	4	*P. expansum*	4	4,06±2,00[a]
5	*R. oryzae*	4	10,62±3,29[a]	5	*R. oryzae*	4	6,25±1,61[a]
6	*F. graminearum*	4	10,0±3,39[a]	6	*F. graminearum*	4	8,75±2,39[a]
7	*S. cerevisiae*	4	12,5±4,45[a]	7	*S. cerevisiae*	4	9,06±3,51[a]
8	*C.rugosa*	4	10,00±2,89[a]	8	*C.rugosa*	4	7,81±2,99[a]

Means for groups in homogeneous subsets are displayed in same letters at 0.05 level (Duncan), p< 0.05, significant). F =02,635, p = 0.831

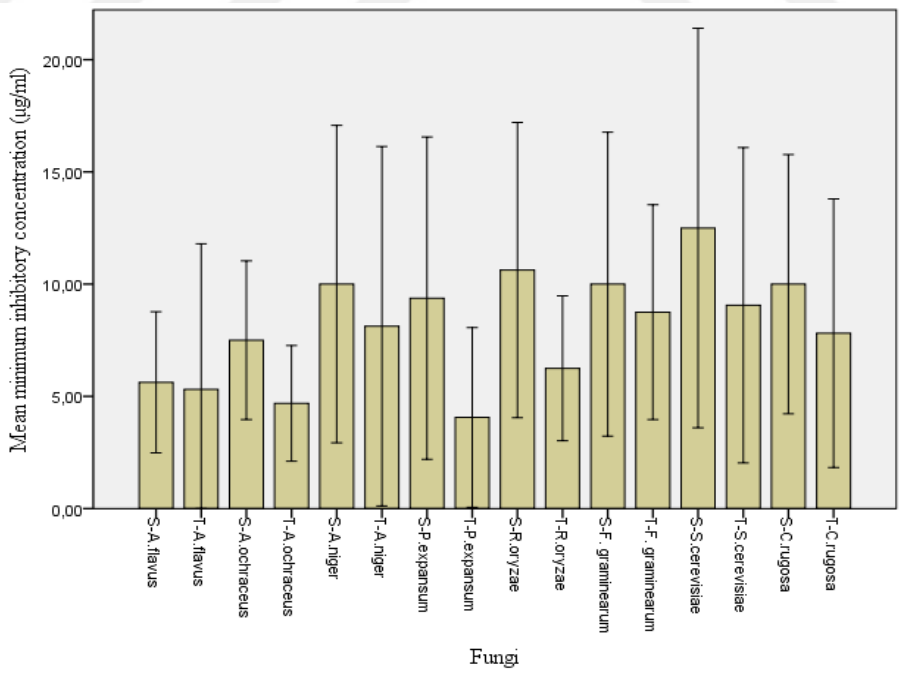

Figure 4.36. Mean MIC values of essential oils of spices from Syria and Turkey against fungi (first fungi are with Syrian essential oils and the Second fungi are with Turkish essential oils)

Comparison of MIC values for fungi according to regional effect is shown in Table 4.13. There isn't any significant difference ($p>0.05$) between Syria and Turkey according to MIC values of essential oils. This means of all MIC values are not significantly different between the two regions. There are several differences between fungi species but when we handle all the date together this result shows us Turkey and Syria plants have similar MIC values.

Table 4.13. Mean MBC values (µg/ml) comparison between Syria and Turkey essential oils with the "Independent- Samples t-Test" for fungi

Country	n	Mean	t	p
Syria	32	9,45±1,05	1,926	0,059
Turkey	32	6,76±0,92		

Essential oils from Syria and Turkey of their MFC values compared with one-way ANOVA and subsets are determined by using Duncan post-hoc tests. Table 4.14. shows that thyme essential oils from Turkey and basil essential oils from Syria and Turkey have significantly lower ($p< 0.05$) MBC values than other plant essential oils on selected fungi.

Table 4.14. Mean MFC values comparison between essential oils from Turkey and Syria with the "One-Way ANOVA" and "Independent samples t-test" for fungi

Essential oil	n	Mean	Essential oil	n	Mean
Essential oil from Syria from Syria			**Essential oil from Syria from Turkey**		
Thyme	8	$4,69±0,57^{ab}$	Thyme	8	$2,97±1,76^{a}$
Mint	8	$10,62±4,77^{cd}$	Mint	8	$7,50±5,18^{bc}$
Chamomile	8	$5,62±0,63^{ab}$	Chamomile	8	$4,06±2,57^{ab}$
Basil	8	$16,88±4,58^{d}$	Basil	8	$12,5±4,63^{d}$

Means for groups in homogeneous subsets are displayed in same letters at 0.05 level (Duncan), $p< 0.05$. (significant). $F = 13,926$, $p = 0.0001$

Mean plot graph Figure 4.37 shows that thyme essential oils have significantly different ($p<0.05$) MFC values than all other plant essential oils for fungi. However,

mean MIC values obtained using Basil from Syria has significantly higher ($p < 0.05$) than others.

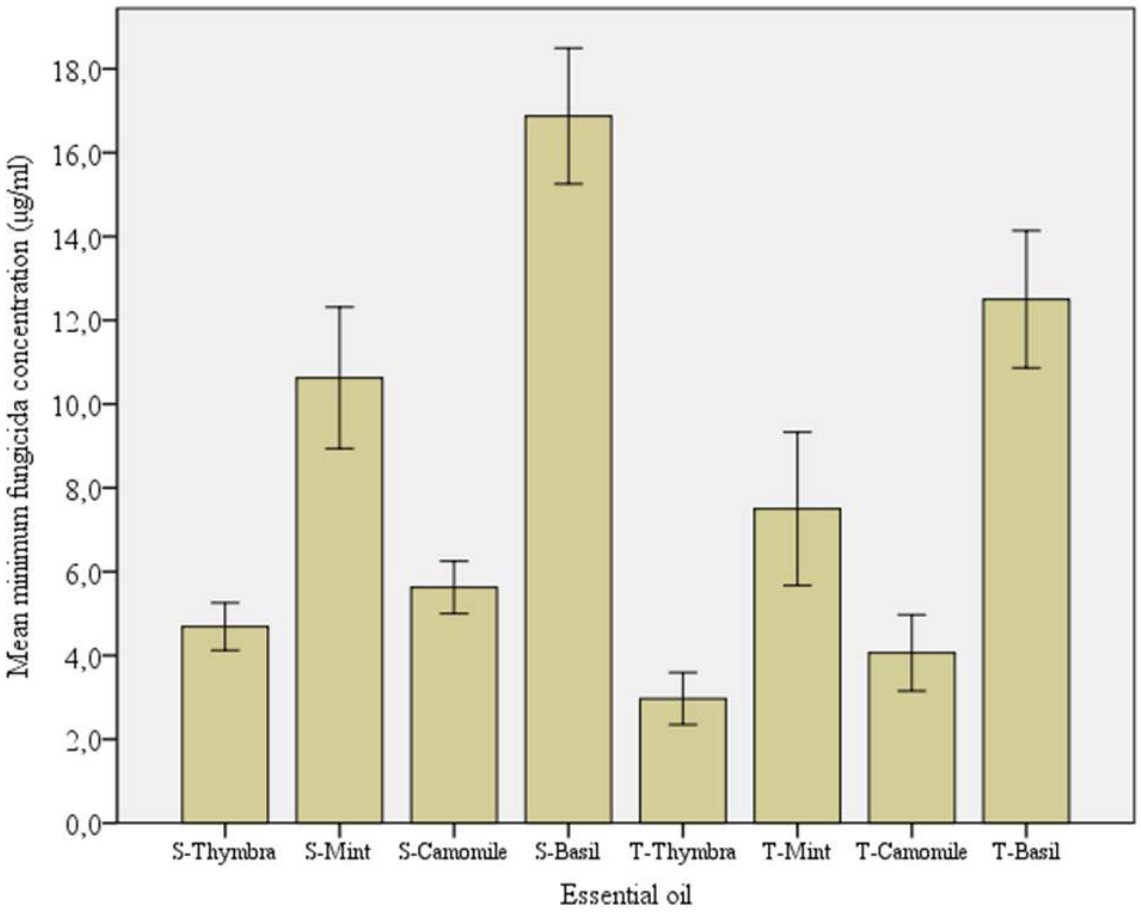

Figure 4.37. Mean MFC values of essential oils from Syria and Turkey for fungi

Table 4.15. shows that there is no significant difference ($p > 0.05$) between Syria and Turkey according to MFC values. As seen, thyme essential oils have significantly different MFC values ($p < 0.05$) than all other plant essential oils. But differences in values clearly shown in Figure 4.38. *A. ochraceus*, *A. flavus* and *P. expansum* need a lower amount of mean essential oils from Syria and Turkey than others.

Table 4.15. Mean MFC values comparison essential oils from Turkey and Syria against fungi with the "One-Way ANOVA" and "Independent samples t-test"

	Essential oil from Syria from Syria				**Essential oil from Syria from Turkey**		
	Fungi	**n**	**Mean**		**Fungi**	**n**	**Mean**
1	*A. flavus*	4	10,00±3,38[a]	1	*A. flavus*	4	5,31±3,24[a]
2	*A. ochraceus*	4	67,52±1,77[a]	2	*A. ochraceus*	4	4,69±2,58[a]
3	*A. niger*	4	10,00±3,54[a]	3	*A. niger*	4	8,12±4,00[a]
4	*P. expansum*	4	9,38±3,59[a]	4	*P. expansum*	4	4,06±2,00[a]
5	*R. oryzae*	4	10,,62±3,29[a]	5	*R. oryzae*	4	6,25±1,61[a]
6	*F. graminearum*	4	10,0±3,39[a]	6	*F. graminearum*	4	8,75±2,39[a]
7	*S. cerevisiae*	4	12,5±4,45[a]	7	*S. cerevisiae*	4	9,06±3,51[a]
8	*C.rugosa*	4	10,00±2,89[a]	8	*C.rugosa*	4	7,81±2,99[a]

Means for groups in homogeneous subsets are displayed in same letters at 0.05 level (Duncan). p< 0.05, significant). F =02,635, p = 0.831

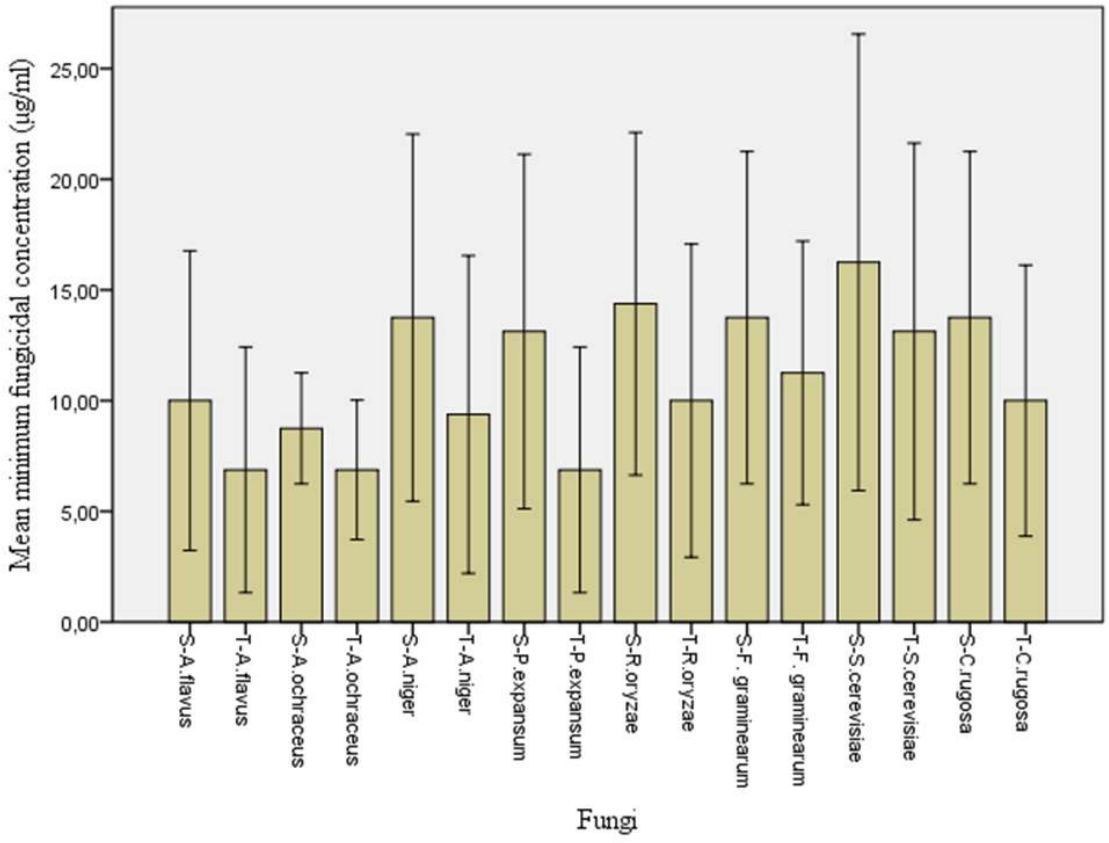

Figure 4.38. Mean MFC values of essential oils of spices from Syria and Turkey against fungi

CHAPTER 5

CONCLUSIONS

- The Turkish and Syrian essential oils have similar MIC values although there are many differences between the studied microorganisms (bacteria, molds, and yeasts).
- The Turkish and Syrian have a more antimicrobial effect on selected microorganisms than other species essential oils and the Syrian basil has higher MIC means than all other plants.
- By comparing between the Gram-positive and negative; The Gram-positive bacteria are less resistant than Gram-negative bacteria because its cells have an outer membrane that is not present in Gram-positive bacteria and have thicker cell wall structure than Gram-negative bacteria.
- The Turkish and Syrian thyme essential oils are more effective on bacterial than other plant essential oils.
- Thyme essential oils regardless of the collected region have an impact and effect on both types of bacteria.
- The extracted essential oils from the neighbor cities such as Gaziantep in Turkey and Aleppo and Idleb in Syria have similar compounds so, they have a similar antimicrobial effect.
- Turkish chamomile and basil showed significantly different ($p<0.05$) effect on selected bacteria due to different climates of regions losing effective essential oils.
- The antimicrobial priorities of the aromatic plants have an important role to enhance the efforts that will support the food and medicine industries.

REFERENCES

Abdulhmid, G., Ana M. Džamić, Marina, S., Mihailo S. Ristić and Petar D. Marin, (2011), Antimicrobial and Antioxidant Activities of Essential Oils of Satureja thymbra Growing Wild in Libya, *Molecules*.***17***, 4836-4850

Alali, W. Q., Hofacre, C. L., Mathis G. F., Faltys,G. (2013). Effect of essential oil compound on shedding and colonization of Salmonella enterica serovar Heidelberg in broilers. *Poult Sci.* **92**, 836–41.

Alvarez, M. V., Ortega-Ramirez, L. A., Gutierrez-Pacheco, M. M., Bernal-Mercado A. T., Rodriguez-Garcia I., Gonzalez-Aguilar, G. A., et al(2014). Oregano essential oil-pectin edible films as anti-quorum sensing and food antimicrobial agents. *Front Microbiol.* **5**,699.10.3389.

Arrebola, M. L., Navarro, M. C., Jiménez J, Ocaña, F. A.(1994). Yield and composition of the essentialoil of Thymus serpylloides subsp. serpylloides. *Phytochemistry*. **36**, 67–72.

Bajpai, V. K., Sharma, A., Baek, K. H., Antibacterial mode of action of Ginkgo biloba leaf essential oil(2015).Effect on morphology and membrane permeability. *Bangladesh J Pharmacol.* **10**,337–50.

Basheer, A., Al-Sum, Abdullah, A., Al-Arfaj, (2013), Antimicrobial activity of the aqueous.

Ben Arfa, A., Combes, S., Preziosi-Belloy, L., Gontard, N., Chalier, P.(2006). Antimicrobial activities of carvacrol related to its chemical structure. *Lett Appl Microbiol.* **43**,149–54.

Benchaar, C., Chaves, A, V., Fraser, G. R., Wang, Y., Beauchemin, K, A., McAllister, T. A.(2007). Effects of essential oils and their components on in vitro rumen microbial fermentation. *Can J Anim Sci.* **87**, 413–9.

Betancourt, L., Rodriguez, F., Phandanouvong, V., Ariza-Nieto, C., Hume, M., Nisbet, D., et al.(2014). Effect ofOriganum chemotypes on broiler intestinal bacteria. *Poult Sci,* **93,** 2526–35.

Bouhdid, S., Abrini, J., Amensour, M., Zhiri, A., Espuny, M. J., Manresa, A.(2010). Functional and ultrastructural changes in Pseudomonas aeruginosa and Staphylococcus aureus cells induced by Cinnamomum verumessential oil. *J Appl Microbiol*. **109**,1139–49.

Bouhdid, S., Abrini, J., Zhiri, A., Espuny, M. J., Manresa, A.(2009). Investigation of functional and morphological changes in Pseudomonas aeruginosa and Staphylococcus aureus cells induced by Origanum compactumessential oil. *J Appl Microbiol*. **106**,1558–68.

Bounatirou, S. S., Miguel, M.G., Faleiro, L., Rejeb, M.N., Neffati, M., Costa, M.M., Figueiredo, A.C., Barroso, J.G., Pedro, L.G. (2007). Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian Thymus capitatus Hoff. et Link. *Food Chemistry.***105**, 146–155.

Boyle, W.,(1955). Spices and essential oils as preservatives. *Am Perfum Essent Oil Rev.* **66**, 25–8.

Callaway, T. R., Carroll, J. A., Arthington, J. D., Edrington, T. S., Anderson, R. C., Rossman, M. L., et al.(2011). Orange-peel products can reduce Salmonella populations in ruminants. *Foodborne Pathog Dis,* **8**,1071–5.

Callaway, T. R., Carroll, J.A., Arthington, J. D., Pratt, C., Edrington, T. S., Anderson, R. C., et al.(2008). Citrus products decrease growth of E. coli O157:H7 and Salmonella Typhimurium in pure culture and in fermentation with mixed ruminal microorganisms in vitro. *Foodborne Pathog Dis.* **5**, 621–7.

Carson, C. F., Mee, B. J., Riley, T. V.(2002). Mechanism of action of Melaleuca alternifolia (tea tree) oil onStaphylococcus aureus determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. *Antimicrob Agents Chemother.* **46**,1914–20.

Cerisuelo, A., Marin, C., Sanchez-Vizcaino, F., Gomez, E. A., de la Fuente, J. M., Duran, R., et al.(2014). The impact of a specific blend of essential oil components and sodium butyrate in feed on growth performance andSalmonella counts in experimentally challenged broilers. *Poult Sci.* **93**, 599–606.

Cox, S. D., Gustafson, J. E., Mann, C. M., Markham, J. L., Liew, Y. C., Hartland, R. P.(1998). Tea tree oil causes K+ leakage and inhibits respiration in Escherichia coli. *Lett Appl Microbiol.* **26**, 355–8.

Cox, S. D., Mann, C. M., Markham, J. L., Bell, H. C., Gustafson, J. E., Warmington, J. R.(2000). The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil). J *Appl Microbiol.* **88**,170–5.

Cox, S. D., Mann, C. M., Markham, J. L., Gustafson, J. E., Warmington, J. R., Wyllie, S. G.(2001). Determining the antimicrobial actions of tea tree oil. *Molecules.* **6,** 87–91.

De Clerck, L. S., Bridts, C. H., Mertens, A. M., Moens, M. M., Stevens, W. J.(1994). Use of fluorescent dyes in the determination of adherence of human leucocytes to endothelial cells and the effect of fluorochromes on cellular function. J *Immunol Methods.* **172**,115–24.

De Souza, E. L., De Azeredo, G. A., De Sousa, J. P., De Figueiredo, R. C. B. Q., Stamford TLM.(2013). Cytotoxic effects of Origanum vulgare L. and Rosmarinus officinalis L. essential oils alone and combined at sublethal amounts on Pseudomonas fluorescens in a vegetable broth. J *Food Safety.* **33**,163–71.

Deans, S. G., Ritchie, G.(1987). Antibacterial properties of plant essential oils. Int J *Food Microbiol.* **5**,165–180.

Deep, A., Chaudhary, U., Gupta, V.(2011). Quorum sensing and bacterial pathogenicity: from molecules to disease. J *Lab Physicians*. **3**, 4–11.

Dorman, H. J. D., Deans, S. G.(2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J *Appl Microbiol.* **88**,308–16.

Erkmen, O. (2015). Basic Methods for the Microbiological Analysis of Foods. *Nobel Academic Publishing Education Consultancy Trade Co*. **3**, 9944-77-055-8, 564.

Erkmen, O., Ozcan, M. (2003).The effects of essential oils of Turkish plantsspices on microorganisms in broth. J. of *Essential Oil-Bearing Plants.***6(1)**,130-134.

Fei Lv, a., Hao Liang a., Qipeng Yuan a., Chunfang, L. i. (2011). In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms, *ELSEVIER,Food Research International.***44**, 3057–3064.

Fisher, K., Phillips, C.(2009). The mechanism of action of a citrus oil blend against Enterococcus faecium andEnterococcus faecalis. J *Appl Microbiol.* **106**, 1343–9.

Fitzgerald, D. J., Stratford, M., Gasson, M. J, Ueckert, J., Bos, A., Narbad, A. (2004). Mode of antimicrobial action of vanillin against Escherichia coli, Lactobacillus plantarum and Listeria innocua. J *Appl Microbiol.* **97**, 104–13.

Fraenkel,G. S.(1959). The raison d'etre of secondary plant substances. *Science*. **129**,1466–70.

Gann, L.D. (2013). Antimicrobial Activity of Essential Oils and Their Components Against Lactic Acid Bacteria. MSc. Thesis, The University of Tennessee, Knoxville.

Gill, A. O., Holley, R. A.(2006). Disruption of E. coli, Listeria monocytogenes and Lactobacillus sakei cellular membranes by plant oil aromatics. Int J *Food Microbiol*. **108**,1–9.

Gill, A. O., Holley, R. A.(2004). Mechanisms of bactericidal action of cinnamaldehyde against Listeria monocytogenes and of eugenol against L. monocytogenes and Lactobacillus sakei. *Appl Environ Microbiol.***70**, 5750–5.

Giweli, A., Džamić, A.M., Soković, M., Ristić, M.S., Marin, P.D. (2012). Antimicrobial and antioxidant activities of essential oils of Satureja thymbra growing wild in Libya. *Molecules*.**17**, 4836-4850

Hammer, K. A., Carson, C. F., Riley, T. V.(1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiol.* **86**, 985–990.

Helander, I. M., Alakomi, H. L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid EJ, et al.(1998). Characterization of the action of selected essential oil components on gram-negative bacteria. J *Agric Food Chem.* **46**, 3590–5.10.

Ifesan, B. O. T., Joycharat, N., Voravuthikunchai, S. P.(2009). The mode of anti-staphylococcal action of Eleutherine americana. *FEMS Immunol MedMicrobiol*. **57**, 193–201.

Inoue, Y., Shiraishi, A., Hada, T., Hirose, K., Hamashima, H., Shimada, J.(2004). The antibacterial effects of terpene alcohols on Staphylococcus aureus and their mode of action. *FEMS Microbiol Lett*. **237**, 325–31.

Kalemba, D., Matla, M., Smetek, A.(2009). Antimicrobial activities of essential oils. Dietary Phytochemicals and Microbes. Berlin: *Springer*; (2012). p. 157–83.

Khan, M. S. A., Zahin, M., Hasan, S., Husain, F. M., Ahmad, I., Inhibition of quorum sensing regulated bacterial functions by plant essential oils with special reference to clove oil. *Lett Appl Microbiol.* **49**, 354–60.

Kjelleberg, S., Molin, S.(2002). Is there a role for quorum sensing signals in bacterial biofilms? *Curr Opin Microbiol*. **5**, 254–8.

Lagouri, V., Blekas, G., Tsimidou, M., Kokkini, S., Boskou, D. (1993). Composition and antioxidant activity of essential oils from oregano plants grown wild in Greece. *Eur Food Res Technol.* **197**, 20–3.

Lambert, R. J. W., Skandamis. P. N., Coote, P. J., Nychas, G. J. E.(2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J *Appl Microbiol.* **91**,453–62.

Lee, S. H., Lillehoj. H. S., Lillehoj. E. P., Cho, S. M., Park, D. W., Hong, Y. H., et al.(2008). Immunomodulatory properties of dietary plum on coccidiosis. *Comp Immunol Microbiol Infect Dis*. **31**,389–402.

Lillehoj, H. S., Kim, D. K., Bravo, D. M., Lee, S. H. (2011). Effects of dietary plant-derived phytonutrients on the genome-wide profiles and coccidiosis resistance in the broiler chickens. *BMC Proc.* **5**,1753-6561-5-S4-S34.

March. J., Bentley, W.(2004). Quorum sensing and bacterial crosstalk in biotechnology. *Curr Opin Biotechnol.***15**, 495–502.

Mathlouthi, N., Bouzaienne, T., Oueslati, I., Recoquillay. F., Hamdi, M., Urdaci, M., et al.(2012). Use of rosemary, oregano, and a commercial blend of essential oils in broiler chickens: in vitro antimicrobial activities and effects on growth performance. J *Anim Sci.* **90**, 813–23.

Miller, M. B., Bassler, B. L.(2001). Quorum sensing in bacteria. *Annu Rev Microbiol.* 55,165–99.

Mith, H., Duré, R., Delcenserie, V., Zhiri, A., Daube, G., Clinquart, A. (2014). Antimicrobial activities of commercial essential oils and their components against food-bornepathogens and food spoilage bacteria. *Food Science and Nutrition*. **2(4)**, 403-416.

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., De Feo, V.(2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals.* **6**,1451–74.

Nguefack, J., Budde, B. B., Jakobsen, M.(2004). Five essential oils from aromatic plants of Cameroon: their antibacterial activity and ability to permeabilize the cytoplasmic membrane of Listeria innocua examined by flow cytometry. Lett *Appl Microbiol.* **39**, 395–400.

Nikaido, H.(2003). Molecular basis of bacterial outer membrane permeability revisited. *Microbiol Mol BiolRev.***67**, 593–656.

Nikaido,H,(1994). Prevention of drug access to bacterial targets: permeability barriers and active efflux. *Science.***264**, 382–8.

Nilima, T., Silpi, B., Rakesh, N., Bahadure3, Monali Rajurkar2, (2013), Antimicrobial efficacy of five essential oils against oral pathogens: An in vitro study, *European Journal of Dentistry*. **7**, 71-7.

Ohmizo, C., Yata, M., Katsu, T.(2004). Bacterial cytoplasmic membrane permeability assay using ion-selective electrodes. J *Microbiol Methods.* **59**, 173–9.

Oussalah, M., Caillet, S., Lacroix, M.(2006). Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of Escherichia coli O157:H7 and Listeria monocytogenes. J *Food Prot.* **69**,1046–55.

Oussalah, M., Caillet, S., Saucier, L., Lacroix, M.(2007). Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157:H7, Salmonella Typhimurium, Staphylococcus aureus andListeria monocytogenes. *Food Control.* **18**, 414–20.

Plesiat, P., Nikaido, H.(1992). Outer membranes of Gram-negative bacteria are permeable to steroid probes. *Molec Microbiol.* **6**,1323–33.

Ricke, S. C., Rivera, C. J., Kaldhone, P.(2015). Salmonella control in food production: current issues and perspectives in the United States. In: Ricke SC, Donaldson JR, Phillips CA, editors., editors. Food Safety Emerging Issues, Technologies and Systems. London: *Academic Press*. 107–33.

Sánchez, E., García, S., Heredia, N.(2010). Extracts of edible and medicinal plants damage membranes of Vibrio cholerae. Appl *Environ Microbiol.* **76**, 6888–94

Sara, B. (2004), Essential oils: their antibacterial properties and potential applications in foods—a review, *International Journal of Food Microbiology.* ***94,***223– 253.

Sartoratto, A., Machado, A., L.M., Delarmelina, C., Figueira, G.M., Duarte, M.C.T.

Schifferstein H. N. J., Ophuis, P. A. M. O.(1998). Health-related determinants of organic food consumption in the Netherlands. *Food Qual Prefer.* **9**,119–33.

Seyyednejad, S.M., Motamedi, H., Vafei, M., Bakhtiari, A. (2014). The antibacterial activity of Cassia fistula organic extracts. *Jundishapur Journal of Microbiology*.**7(1),** 8921.

Sikkema, J., de Bont, J. A. M., Poolman, B.(1994). Interactions of cyclic hydrocarbons with biological membranes. J *Biol Chem.* **269**, 8022–8.

Szabo, M. A., Varga, G. Z., Hohmann, J., Schelz, Z., Szegedi, E., Amaral, L., et al. (2010). Inhibition of quorum-sensing signals by essential oils. *Phytother Res.* **24**,782–6.

Tat jana, M., PASCHALINA, C., J. O. vana Š., M. Ni koli ć3, Jasmina, G., Ana Ći rić3, Marina. S. (2011). Chemical Analysis and Antimicrobial Ac tivities of the Essential Oils of Satureja thymbra L. and Thymbra spicata L. and their main components, Arch. Biol. *Sci., Belgrade*.**63 (2),** 457-464.

Thosar, N., Basak, S., Bahadure, R.N., Rajurkar, M. (2013). Antimicrobial efficacy of five essential oils against oral pathogens: An in vitro study. *European Journal of Dentistry.***7**, 71-76.

Togashi, N., Inoue, Y., Hamashima, H., Takano, A.(2008). Effects of two terpene alcohols on the antibacterial activity and the mode of action of farnesol against Staphylococcus aureus. *Molecules.* **13**,3069–76.

Trombetta, D., Castelli, F., Sarpietro. µ. G., Venuti, V., Cristani, M., Daniele, C., et al. (2005). Mechanisms of antibacterial action of three monoterpenes. *Antimicrob Agents Chemother.* **49**,2474–8.

Tuncay. G., Ahmet, S. D., Munteha, N. S., Nazan, T. D., Eray, T., Mehmet,G. (2016). Investigation of antimicrobial and antioxidant activities of essential oils extracted from medicinal plants.*Arch Lebensmittelhyg*.**67**, 17–24.

Turgis, M., Han, J., Caillet, S., Lacroix, M.(2009). Antimicrobial activity of mustard essential oil againstEscherichia coli O157:H7 and Salmonella typhi. *Food Control.* **20**,1073–9.

Ultee, A., Bennik, M. H. J., Moezelarr, R.(2002). The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen Bacillus cereus. *Appl Environ Microbiol.* **68,**1561–8.

Ultee, A., Kets, E. P. W., Alberda, M., Hoekstra, F. A., Smid, E. J. (2000). Adaptation of the food-borne pathogenBacillus cereus to carvacrol. *Arch Microbiol.* **174**, 233–8.

Ultee, A., Kets, E. P. W., Smid, E. J.(1999). Mechanisms of action of carvacrol on the food-borne pathogens Bacillus cereus. *Appl Environ Microbiol.* **65**,4606–10.

Veal, D. A., Deere, D., Ferrari, B., Piper, J., Attfield, P. V.(2000). Fluorescence staining and flow cytometry for monitoring microbial cells. J *Immunol Methods*. **243,**191–210.

Veldhuizen, E. J. A., Tjeerdsma, V., Bokhoven, J. L. M., Zweijtzer, C., Burt, S. A., Haagsman, H. P.(2006). Structural requirements for the antimicrobial activity of carvacrol. J *Agric Food Chem.* **54**,1874–9.

Walsh, S. E., Maillard, J. Y., Russell, A. D., Catrenich, C. E., Charbonneau, D, L., Bartolo, R. G.(2003). Activity and mechanisms of action of selected biocidal agents on gram-positive and –negative bacteria. J *Appl Microbiol.***94**, 240–7.

Williams, P., Stirling, E., Keynes, N.(2004). A national survey on the attitudes of Australian adults about the safety and quality of food. Asia Pac J *Clin Nutr.* **13**, 32–9.

Williams. P. R. D., Hammitt, J. K.(2001). Perceived risks of conventional and organic produce: pesticides, pathogens, and natural toxins. *Risk Anal.* **21**,319–30.

Xu, J., Zhou, F., Ji, B. P., Pei, R. S., Xu, N.(2008). The antibacterial mechanism of carvacrol and thymol againstEscherichia coli. *Lett Appl Microbiol.* **47**, 174–9.

Appendix

Table A.1. The antimicrobial effects of essential oil (µg/ml) MIC obtained from Syria and Turkey species on pathogen and spoilage bacteria

Bacteria	**Essential oil from Syria species**					**Essential oil from Turkey species**				
	Thymbra spicata (thyme)	*Pimpinella anisum* (anise)	*Folium menthae* (mint)	*Matricaria chamomilla* (chamomile)	*Ocimum basilicum* (basil)	*Thymbra spicata* (thyme)	*Pimpinella anisum* (anise)	*Matricaria chamomilla* (chamomile)	*Folium menthae (mint)*	*Ocimum basilicum* (basil)
Escherichia coli	0,5	25	20	30	20	0,25	10	20	25	7,5
Salmonella Typhimurium	0,25	30	35	10	50	0,25	25	7,5	20	40
Yercinia enterocolitica	0,25	20	40	10	50	0,1	10	10	25	40
Pseudomonas aeroginosa	0,25	35	30	10	50	0,25	30	5	15	35
Shigella dysanteriae	0,25	50	30	2,5	40	0,25	45	2,5	20	35
Klebsiella pneumoniae	1,25	60	90	125	60	1,25	40	100	75	50
Listeria monocytogenes	1,25	45	30	5	35	0,75	40	5	20	30
Staphylococcus aureus	0,75	10	25	20	40	0,25	5	15	20	35
Bacillus cereus	1	70	40	25	60	0,5	50	15	30	60
Bacillus subtilis	0,5	50	40	20	50	0,25	45	15	20	50
Enterococcus faecalis	0,75	30	25	15	40	0,75	25	10	25	30
Streptococcus thermophilus	0,25	20	20	7,5	10	0,5	15	5	10	7,5

Table A.2. The antimicrobial effects of essential oil (μg/ml) MBC obtained from Syria and Turkey species on pathogen and spoilage bacteria

Bacteria	**Essential oil from Syriaspecies**					**Essential oil from Turkey species**				
	Thymbra spicata (thyme)	*Pimpinella anisum* (anise)	*Folium menthae* (mint)	*Matricaria chamomilla* (chamomile)	*Ocimum basilicum* (basil)	*Thymbra spicata* (thyme)	*Pimpinella anisum* (anise)	*Folium menthae* (mint)	*Matricaria chamomilla* (chamomile)	*Ocimum basilicum* (basil)
Escherichia coli	0,75	30	25	35	30	0,75	25	35	25	12,5
Salmonella Typhimurium	0,75	40	40	20	60	0,5	35	30	15	50
Yercinia enterocolitica	0,5	20	40	20	65	0,25	10	30	15	50
Pseudomonas aeroginosa	0,5	45	35	15	55	0,25	40	25	15	50
Shigella dysanteriae	0,5	60	40	5	50	0,25	50	25	2,5	40
Klebsiella pneumoniae	1,75	80	100	130	75	1,5	75	85	125	60
Listeria monocytogenes	2,5	50	40	10	50	1	50	25	7,5	40
Staphylococcus aureus	1	15	30	25	40	0,5	10	25	20	40
Bacillus cereus	1,5	80	45	30	70	1	65	40	20	65
Bacillus subtilis	1,25	70	45	25	60	0,5	50	30	20	50
Enterococcus faecalis	1,25	35	35	20	45	1	30	30	20	40
Streptococcus thermophilus	0,5	25	30	10	15	0,75	20	20	7,5	10

Table A.3. The antimicrobial effects of essential oil (µg/ml) MTC obtained from Syria and Turkey species on pathogen and spoilage bacteria

Bacteria	**Essential oil from Syria species**					**Essential oil from Turkey species**				
	Thymbra spicata (thyme)	*Pimpinella anisum* (anise)	*Folium menthae* (mint)	*Matricaria chamomilla* (chamomile)	*Ocimum basilicum* (basil)	*Thymbra spicata* (thyme)	*Pimpinella anisum* (anise)	*Folium menthae* (mint)	*Matricaria chamomilla* (chamomile)	*Ocimum basilicum* (basil)
Escherichia coli	0,25	20	15	25	15	0,1	7,5	20	20	5
Salmonella Typhimurium	0,1	25	30	7,5	40	0,1	20	15	5	35
Yerciniaenterocolitica	0,1	15	35	7,5	40	0,05	7,5	20	7,5	35
Pseudomonas aeroginosa	0,1	30	25	7,5	40	0,25	25	10	2,5	50
Shigella dysanteriae	0,1	45	25	1,25	30	0,1	40	15	1,25	30
Klebsiella pneumoniae	10	45	75	90	55	1	35	65	80	45
Listeria monocytogenes	1	40	25	2,5	30	0,5	35	15	2,25	25
Staphylococcus aureus	0,5	5	20	15	35	0,1	2,5	15	10	30
Bacillus cereus	0,75	55	35	20	55	0,25	45	20	10	55
Bacillus subtilis	0,25	45	35	15	45	0,1	40	15	10	40
Enterococcus faecalis	0,5	25	20	10	35	0,5	20	20	7,5	25
Streptococcus thermophilus	0,1	15	15	5	7,5	0,1	10	7,5	2,5	5

Table A.4. The antimicrobial effects of essential oil (µg/ml) MIC obtained from Syria and Turkey species on toxigenic and spoilage fungi

Fungi	Essential oil from Syria species				Essential oil from Turkey species			
	Thymbra spicata (thyme)	*Folium menthae (mint)*	*Matricaria chamomilla* (chamomile)	*Ocimum basilicum* (basil)	*Thymbra spicata* (thyme)	*Folium menthae (mint)*	*Matricaria chamomilla* (chamomile)	*Ocimum basilicum* (basil)
Saccharomyces cerevisiae	5	7,5	2,5	20	2,5	5	1,25	15
Candida rugosa	5	10	5	15	2,5	7,5	1,25	10
Aspergillus flavus	2,5	5	5	10	1,25	2,5	2,5	15
Aspergillus ochraceus	2,5	10	7,5	10	1,25	5	5	5
Aspergillus niger	5	10	5	20	2,5	5	5	20
Penicillium expansum	5	7,5	5	20	1,25	2,5	2,5	10
Rhizopus oryzae	5	10	5	20	2,5	5	2,5	10
Fusarium graminearum	5	7,5	5	20	5	10	2,5	15

Table A.5. The essential oil (µg/ml) MFC values obtained from Syria and Turkey species on toxigenic and spoilage fungi

Fungi	Essential oil from Syria species				Essential oil from Turkey species			
	Thymbra spicata (thyme)	*Folium menthae (mint)*	*Matricaria chamomilla* (chamomile)	*Ocimum basilicum* (basil)	*Thymbra spicata* (thyme)	*Folium menthae (mint)*	*Matricaria chamomilla* (chamomile)	*Ocimum basilicum* (basil)
Saccharomyces cerevisiae	7,5	20	7,5	20	5	15	5	15
Candida rugosa	5	15	10	10	5	15	7,5	10
Aspergillus flavus	7,5	15	7,5	25	5	7,5	5	20
Aspergillus ochraceus	10	10	7,5	25	2,5	5	7,5	15
Aspergillus niger	10	15	7,5	25	5	5	10	20
Penicillium expansum	10	10	10	25	7,5	10	7,5	20
Rhizopus oryzae	10	25	7,5	25	10	20	7,5	20
Fusarium graminearum	10	20	7,5	20	7,5	15	5	15

Table A.6. The antimicrobial effects of essential oil (µg/ml) MTC obtained from Syria and Turkey species on toxigenic and spoilage fungi

Fungi	Essential oil from Syria species				Essential oil from Turkey species			
	Thymbra spicata (thyme)	*Folium menthae (mint)*	*Matricaria chamomilla* (chamomile)	*Ocimum basilicum* (basil)	*Thymbra spicata* (thyme)	*Folium menthae (mint)*	*Matricaria chamomilla* (chamomile)	*Ocimum basilicum* (basil)
Saccharomyces cerevisiae	1,25	2,5	2,25	5	0,75	1,25	1,25	10
Candida rugosa	1,25	7,5	5	5	0,75	2,5	5	2,5
Aspergillus flavus	2,5	5	2,5	15	1,25	2,5	2,5	15
Aspergillus ochraceus	2,5	5	2,25	15	0.,5	1,25	1,25	5
Aspergillus niger	2,5	7,5	5	15	1,25	2,5	5	5
Penicillium expansum	2,5	5	5	15	2,5	7,5	2,5	10
Rhizopus oryzae	5	15	1.,5	15	2,5	10	0,75	10
Fusarium graminearum	2,5	10	2,5	10	2,5	10	0,75	5