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Antimicrobial Activity of *Varthemia iphinoides* and *Majorana syriaca* Essential Oils from Jordan and Their Potential Use as Natural Food Preservatives

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Abstract

The aim of this study was to evaluate the efficiency of *Varthemia iphinoides* and *Majorana syriaca* essential oils as natural food preservative in four different food model media (cheese, meat, milk, and tomato) against two gram-positive and two gram-negative bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) and three different food spoilage fungi using agar well diffusion method. The results showed that the maximum activity of *V. iphinoides* essential oil was against *S.aureus* in tomato media with relative inhibition zone diameter (RIZD) 186 ± 9 %, the most sensitive food spoilage fungi was *penicillium* sp. in tomato media with RIZD 149 ± 10 %. The results showed that there was inhibitory effect of *M. syriaca* and *V. iphinoides* essential oils. against most of the tested species in different food model media which will lead to control food pathogen organisms by using essential oils as food preservative and flavoring agents.

Key words: *Varthemia iphinoides , Majorana syriaca,* Essential oils**,** Antimicrobial**,** Food preservatives

1. Introduction

Protection of different food products is needed against microbial spoilage to increase food shelf life. There are growing demands of consumers for safe and natural products, without chemical preservatives. Therefore, there has been increasing interest from researchers to assess the feasibility of mild preservation techniques and to improve the microbial quality and safety of products, and to replace chemical preservatives with natural, effective and nontoxic compounds. (Goñi et al., 2009).

Essential or volatile oils are aromatic oily liquids obtained from any of plant materials (flowers, leaves, roots, fruits and seeds). Essential oils can be obtained by different methods such as expression, fermentation, extraction and steam distillation, but the most commonly used method is steam distillation method which used for commercial production of essential oils (Van de Braak and Leijten, 1999). The essential oils are used in food, perfumes and pharmaceuticals. Also little more than 2% of the total markets are the well-known use of essential oil in aromatherapy (Burt, 2004).

Essential oils and their components have antibacterial properties which exploited in different commercial products such as dental root canal sealers, antiseptics and feed supplements. A few of available preservatives contain essential oils. One of these food preservatives contain rosemary, sage and citrus essential oils (50%) and glycerol (50%) (Mendoza-Yepes et al., 1997). Another safe food additives is Protecta One and Protecta Two which are blended herb extracts produced commercially. Sodium citrate and sodium chloride are used respectively to disperse extracts which probably contain one or more essential oils, although the precise contents are not made known by the manufacturer (Cutter, 2000). Further essential oils effects are using them in different products such as commercial potato bud suppressants and insect repellents (Burt, 2004).

Higher concentrations of essential oils are needed in food to achieve the same effect of essential oil when used against food-borne pathogens and spoilage microorganisms when in vitro tests are conducted (Burt, 2004; Hulin, et al., 1998). Few studies have been reported to reduce essential oil concentrations which will minimize the sensory effect. Essential oils are generally safe, because of reducing their natural antimicrobial effects by the addition of small amounts of other natural preservatives may be possible also may be a way of attaining a balance between sensory acceptability and antimicrobial efficiency (Goñi et al., 2009).

The aim of the present study was to investigate the potential role of *Majorana syriaca* and *Varthemia iphinoides* essential oils for their antibacterial and antifungal activities using an in vitro different food model media (cheese, meat, and tomato).

2MATERIALS AND METHODS

2.1 Plant material and Essential Oils Extraction

Varthemia iphinoides and *Majorana syriaca* samples were collected from North Badia, Mafraq, Jordan, during April to June 2013. The aerial parts of the collected plant samples were subjected to steam distillation for 3.0 to 4.0h. After decanting, the obtained essential oils were dried over anhydrous sodium sulfate, stored in refrigerator at - 4°C until tested and analyzed.

2.2 Antimicrobial assay of essential oils

In vitro antimicrobial assay of the oils were carried out according to agar wells diffusion method (Ruberto et al., 2000; Burt, 2004). Antimicrobial activity of the oils was tested against gram positive bacterial strains (*Bacillus subtillis* and *Staphylococcus aureus)*, gram negative bacterial strains (*Escherichia coli* and *Pseudomonas aureginosa)*, and three fungal species (*Aspergillus niger*, *Penicillium sp.* and *Mucur sp).* Food model media agar was used as the cultivation medium for bacterial and fungal species. The microbial suspensions were streaked over the surface of food model media agar using a sterile cotton swab to ensure the confluent growth of the organism. The oils were diluted in 5% concentrations using dimethylsulphoxide (DMSO). Aliquots of 100 µl of the diluted oils were added to agar well. The plates were incubated at 37°C for 24 h for bacteria and at 28°C for 72 h for fungi, then inhibition zones diameter were measured. Cephaclor (5 mg /ml) and fluconazole (10mg/ml) were used as positive control for bacteria and fungi respectively. The antimicrobial activity will be calculated by applying the expression:

% RIZD = ((IZD sample – IZD negative control)/ IZD antibiotic standard) × 100 %

(Perez et al. 1990)

Where RIZD is the percentage of relative inhibition zone diameter and IZD is the inhibition zone diameter (mm).

2.3 Food model media preparation

200 g of tomato, meat, handmade cheese and milk were added to 100 ml of sterile deionized water and shaken for 1 min. The suspension was mixed and homogenized by a blinder then filtered using cotton gauze. In order to obtain a solid media, 1.5% agar was added to food model media. The media were then autoclaved at 121ºC for 15 min.

3. Results:

The antibacterial activities of *V. iphinoides* and *M. syriaca* essential oils were given in figures (1) and (2), respectively. The results showed that both essential oils possessed significant antibacterial activity comparing to the standard antibiotic. The essential oils possessed antibacterial activity against all used bacterial strains with all type of food media models (figure 1 & 2). The highest antibacterial activity of *M. syriaca* with relative inhibition zone diameter (RIZD) 186 ± 9 % was observed against *S. aureus* using tomato media, while the essential oil of *V. iphinoides* showed the lowest antibacterial activity with RIZD 67 ± 6% against *P. aureginoza* using meat media.

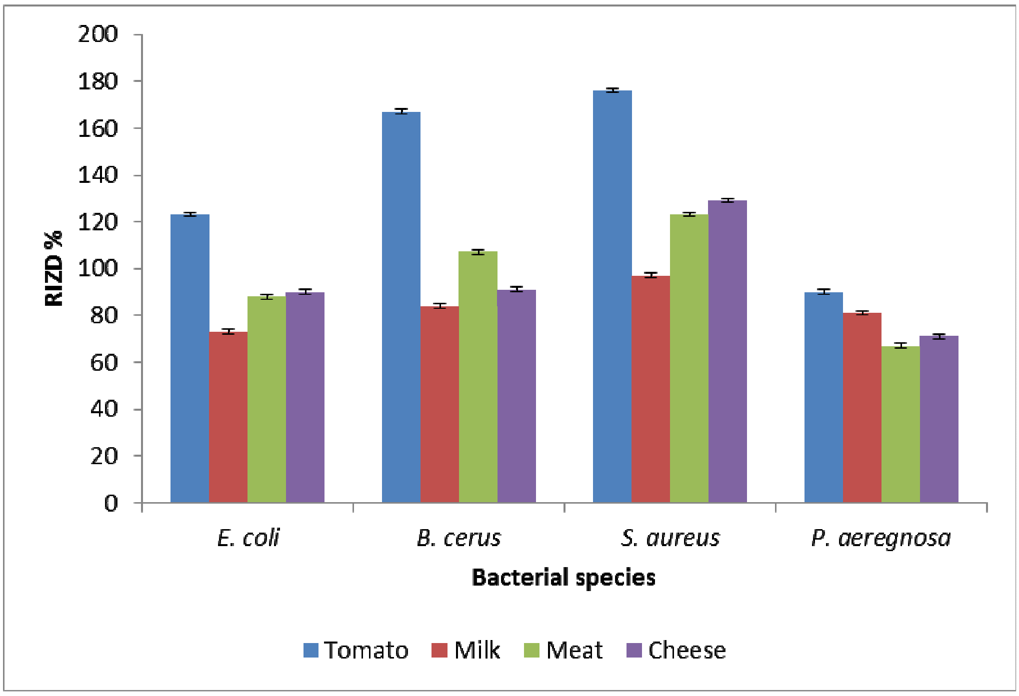

Figure 1: Antibacterial activity (RIZD %) of *V. iphinoides* essential oil against four tested species

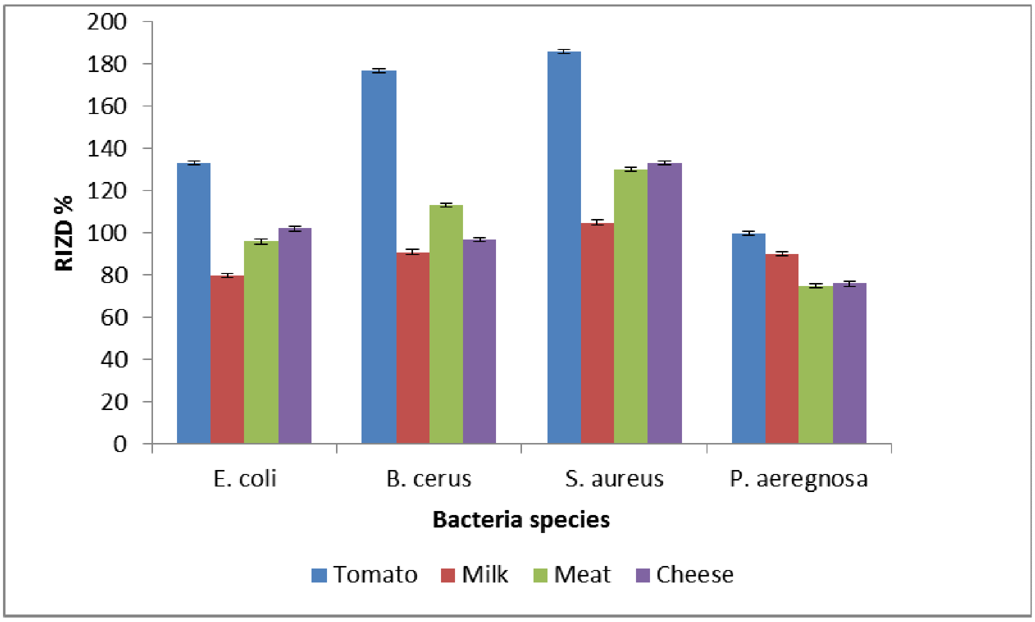

Figure 2: Antibacterial activity (RIZD %) of *M. syriaca* essential oil against four tested species

The antifungal activities of essential oils of *V. iphinoides* and *M. syriaca* are given in figures (3&4), respectively. The results showed that the essential oils of both plants showed significant antifungal activity comparing to the standard antifungal. The antifungal activities of *M. syriaca* essential oil showed the highest RIZD (146± 9 %) using meat model media comparing to standard antifungal , while the essential oil of *V. iphinoides* showed the lowest RIZD (86± 7)% using milk model media.

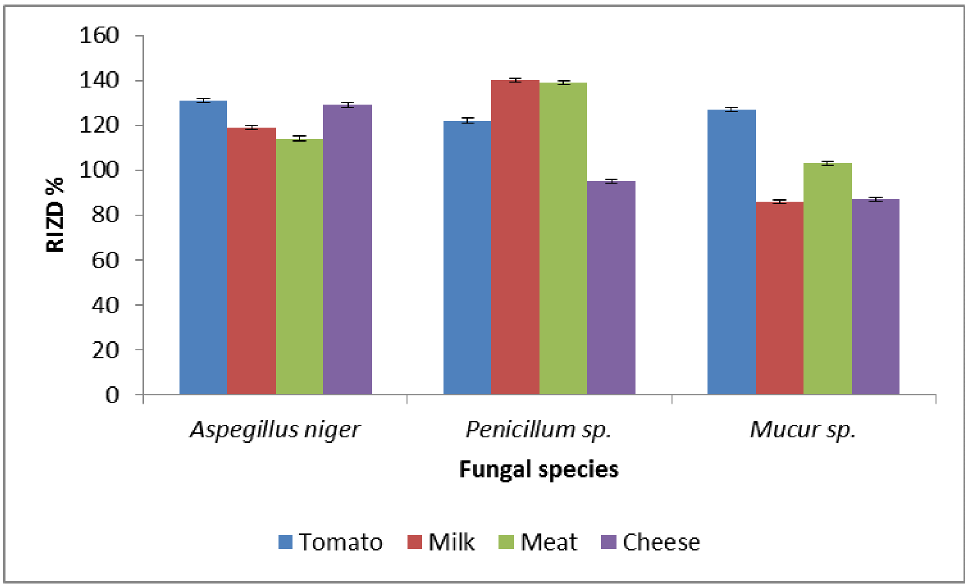

Figure 3: Antifungal activity (RIZD %) of *V. iphinoides* essential oil against three tested species

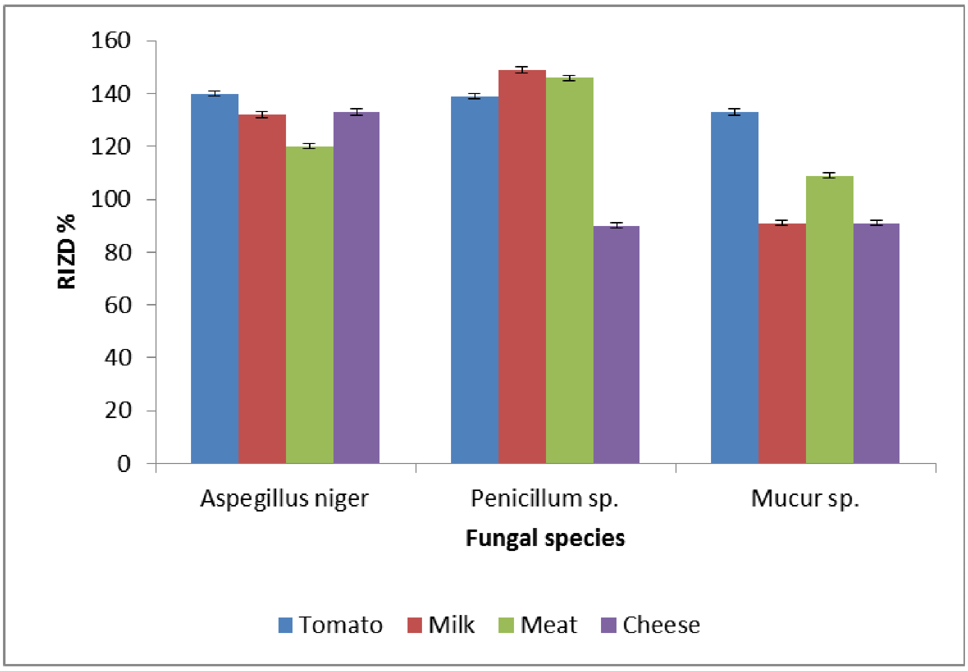

Figure 4: Antifungal activity (RIZD %) of *M. syriaca* essential oil against three tested species

The effect of essential oils of *M. syriaca* and *V. iphinoides* are shown in figures (5&6), respectively. The results showed that the essential oils of both plants showed significant reduction in the percent of germinated spores. Both essential oils inhibit spore more than 80% of germinated spore at 75 µg/ml and 100 µg/ml concentrations, respectively.

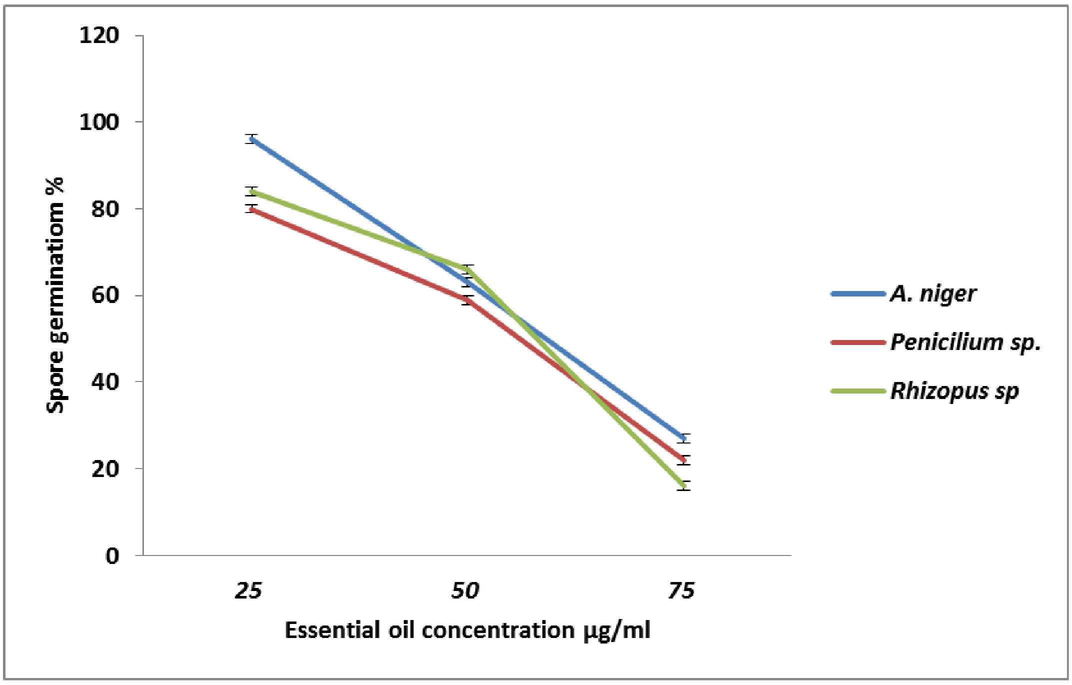

Figure5: Effect of *M. syriaca* essential oil on spore germination of fungi

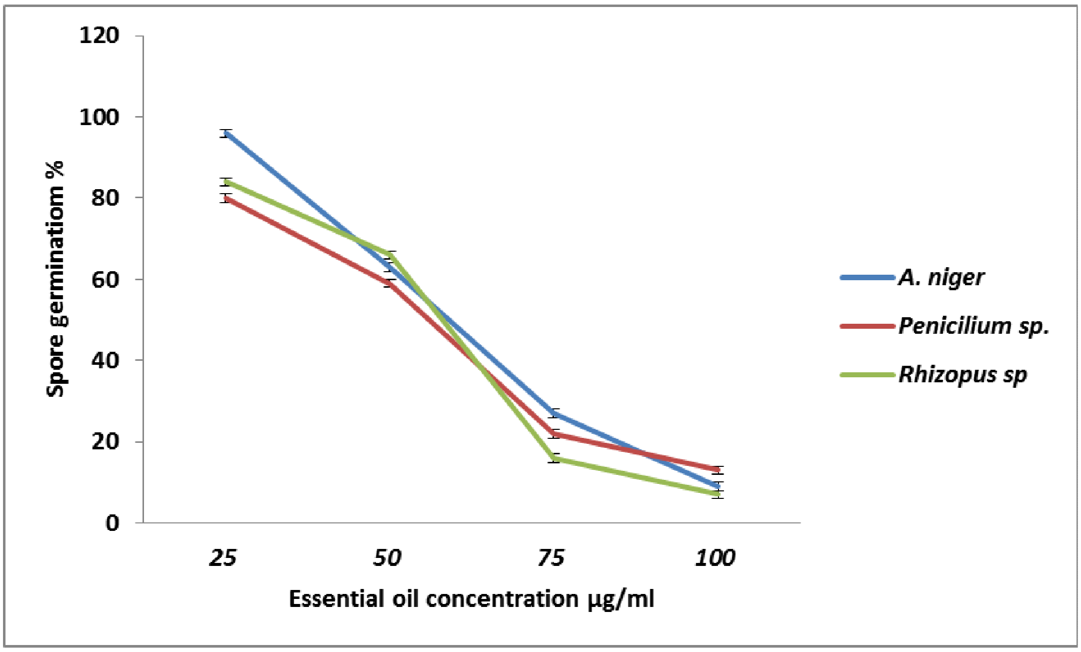

Figure6: Effect of *V. iphinoides* essential oil on Spore germination of fungi

4. Discussion

The antibacterial activity of essential oils was performed against four bacterial species. *E. coli* and *P. auregenosa* are gram negative bacteria, the other two species *B. subtilis* and *S. aureus* are gram positive bacteria. The essential oils (figures 1&2) possessed antibacterial activity and inhibit the growth of all tested bacterial species. The essential oils of both tested plants possessed varied antimicrobial activity comparing to comparing to standard antibiotics. The essential oil showed variation in antibacterial activity against different bacteria species using different type of food model media. The variations in bacterial response to essential oils may refer to the structural difference of bacteria and the mode of action of the essential oils against these bacterial species.

The antifungal activity of *V. iphinoides* essential oil was performed against three fungal species that were isolated from food samples. These species were chosen according to their ability as food contaminants. The tested essential oil (figures3 & 4) possessed variant antifungal activity against the three species and inhibit the growth of all tested fungal species. The effect of essential oils varied also with all food model this variation may refer to the difference in the constituent of each food media which may enhance or alter the growth of fungi.

Comparison between results reported about antibacterial properties of different essential oils is very difficult due to the variation in methods of antimicrobial effects evaluation, source of essential oil and different species of used microbes (Arab and Ettehad, 2008). Several studies have been carried out on antimicrobial effects of plants and they have demonstrated that some few are the most important essential oil components that are responsible for antimicrobial properties. Kim et al. (1995)

The essential oils of *Majorana syriaca* and *Varthemia iphinoides* reduce the percentage of spore germination at concentration dependent manner (figures 5&6). Both essential oils completely inhibit spore germination at different concentration. The inhibition of spore germination was observed to be concentration dependent. These results coup with Udomsilp *et al*. (2009) result about the efficacy tests of two essential oils that undertaken by mycelium growth and spore germination inhibition under in vitro conditions against rice pathogenic fungi. The inhibition of spore germination by low concentration of plant essential oils give the essential oils a good chance to be used for inhibition of fungal growth. These essential oils may replace antifungal drugs due to the fact of the side effect of these drugs.

5. Conclusion

From the above discussion it is cleared that *V. iphinoides* and *M. syriaca* essential oils may be act as promising food preservative due to their high antimicrobial activity against gram positive, gram negative bacteria and fungi. The safe use of these two plant as food or food additive n Jordan may bring the attention toward using their essential oils to avoid side effect of chemical food preservatives. According to the previous results this study highly recommends to isolate pure chemical compound from these essential oils and test their potential antimicrobial activity.

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