Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2012, 3 (12):5674-5676
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Inhibition effect of *Zataria multiflora* essential oil on *Lactobacillus sp.* bacteria in plant tissue culture

Mohammad Reza Nakhzari-Moqadam[1*] and Seyed Kazem Sabbagh[2]

[1]*Department of Horticultural, Science and Research Branch, Islamic Azad University, Sistan and Baluchestan, Zahedan, Iran.*
[2]*Department of Plant Protection and Institute of Plant Biotechnology (Biocenter), University of Zabol, Zabol, Iran*

ABSTRACT

Plant tissue cultures may be contaminated by a wide range of fungi and bacterial species. In order to investigate the effect of Zataria multiflora essential oil to prevent the growth of bacterial contamination on plant tissue culture medium, Z. multiflora essential oil were used at concentrations 100- 1000 ppm in MS medium. Lactobacillus sp. strains are a serious problem in culture medium used in plant tissue culture. The effect of essential oil on bacteria at two concentrations (A: 2 and A: 0.1 – 0.2) separately were analysis. The results showed that the essential oil in 400 ppm concentration increased the rate of bacterial growth inhibition as lowest concentration of bacteria (A: 0.1-0.2) and in 700 ppm concentration as highest concentration (A:2). Our result indicates that the use of essential oils could be considered as a practical method for removing contaminants in plant tissue culture procces.

Keywords: Tissue culture, Thyme's essential oil, bacterial contamination, *Lactobacillus sp.*

INTRODUCTION

Plant tissue culture in vitro methods, including biotechnology techniques have been used since 1950s [1]. That is one of the appropriate and fast ways to replicate many of the plants species. But infection in media is one of the serious problems that the researches are constantly encountered with. Plant tissue culture may be infected by a wide range of bacterial that many of which are species specific. Bacterial contamination can reduce growth rate, retard rooting and even cause plant death [4]. Generally, a bacteria genus (Lactobacillus) as a contamination in medium culture has been found in many plant tissue culture approaches [5]. Essential oils due to antibacterial, antifungal, allelopathic, antioxidant and biological activity have the high defense mechanisms against the pathogens and the pests. Plant thyme (*Zataria multiflora* Boiss) has shown anti-bacterial and anti-oxidant properties due to their phenolic material and aromatic compound [2]. In this study, inhibitory effect of *Zataria multiflora* essential oil on the bacterial genera (*Lactobacillus*) in the culture medium used in plant tissue culture was assayed.

MATERIALS AND METHODS

Isolation of bacteria

The contaminating bacteria were isolated by placing material from visibly contaminated cultures directly on NA (Nutrient Agar) medium then identified as *Lactobacillus sp.The bacteria were stored at -80˚c.* A new culture (24 h) of *Lactobacillus sp.* strain was prepared in NA media. Dilutions of the bacterial suspension was measured by using a spectrophotometer with a wavelength of 600 nm (10^5 CFU/ml) and the suspension of the bacterial cells with various concentrations (A: 0.1- 0.2, 2) was prepared. A bacterial lawn was prepared by spreading a suspension of the bacteria on MS medium containing different levels of thyme essential oil (0, 100, 200 - 1000 ppm). The plates were

incubated for 24 hours at 25°C in the dark. The diameters of the inhibition zones around the disks were measured and recorded.

Essential oil preparation

Zataria multiflora essential oil of 99% purity was provided by Rotary and stored in dark bottles at 4 º C until use. To provide essential oil basic solution of NaOH (1N) was used to dissolve oils and pH of medium was adjusted to 5.8. Five replicates for each treatment were considered and the test was repeated triplet. Data analyses from the experiments were performed by using SPSS software.

RESULTS AND DISCUSSION

The results wide range concentrations showed that the essential oil at a concentration of 400 ppm in the bacterial diluted A: 0.1- 0.2 and concentration of 700 ppm in the bacteria diluted A: 2 cause completely inhibitatin the growth of pollution in the plant cultivation environment (Table 1).

Table 1: The effect of different concentrations of essential oil in inhibition of growth of two dilutions of bacterial

	Concentrations (ppm)						
Dilutions of bacterial	100	200	300	400	500	600	700
A: 0.1- 0.2	-	-	-	+	+	+	+
A: 2	-	-	-	-	-	-	+

+: inhibition of growth the bacterial, -: non inhibition of growth the bacterial

According to the figure 1 and 2 it can be concluded with increasing essential oil concentration, deterrence of develop pollution will be increases, as in 400 and 700 ppm concentrations observe a complete inhibition. These results can be attributed to phenolic compounds. Phenolic compounds present in the essential oils are most effective in providing antimicrobial. These compounds penetrate in cell membrane and also can have been involved in clotting the cell contents [3].

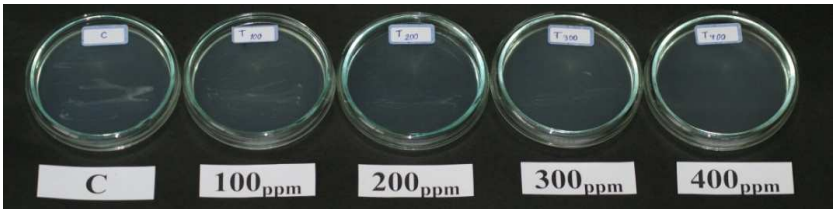

Figure 1: effect of different concentrations of essential oil in inhibition of growth the bacterial diluted A: 0.1- 0.2

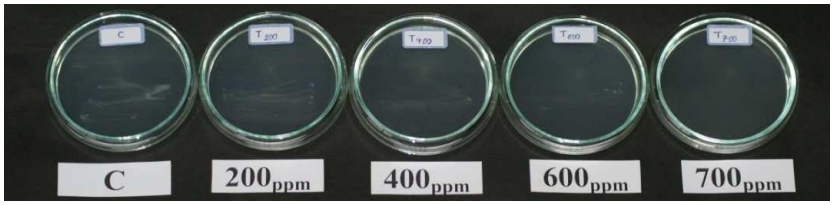

Figure 2: effect of different concentrations of essential oil in inhibition of growth the bacterial diluted A: 2.

Essential oil of *Zataria multiflora* with the concentrations of %1, 2.5 and %5 on two *Salmonella typhimorium* isolates shown that these compound could prevent the growth of 8.6- 9.3 and 15.6- 21.6 mm, respectively [9]. In another experiment, the concentration of *Z. multiflora* essential oil to 200 ml in 20 ml of *Aspergillus parasiticus* fungi reduced diameter colony of fungus on culture medium [6]. The ability of this essential oil to have complete control of *A. parasiticus* has been assayed. Using thyme's essential oil in 400 ppm concentrations showed the highest reduction of *A. parasiticus* fungi in culture medium [6]. The inhibition of bacteria in specific culture medium has been tested on *Escherichia coli*. Essential oil of *Z. multiflora* in 10 µl/ ml concentration reduced 15 mm of diameter of bacterial colony [7]. Our result is in concordance with previous study that has shown the effect of thyms essential oil against bactria grown on culture medium. Instead of using chemical compounds with noxious effects on plant tissue for remove infectious agent including fungi and bacteria, essential oils can be use as an alternative compound to control these contaminations. Although, economically, essential oil in competition with fungicide and bactericide either cheap or easy to manipulation and purification and could be used in low concentrations.

CONCLUSION

In this study the effect of *Z. multiflora* essential oil on growth and development of *Lactobacillus sp*. In contaminated plant tissue culture was investigated. These data show that this component could inhibit diameter and colony number of bacteria on MS medium so we propose the use of this component to prevent of bacterial contamination in plant tissue culture manipulation.

Acknowledgment
We thank Miss Eftekhari to prepare essential oil in institute of plant biotecnolgy of Shahid Beheshti Uinversity. This research is abstraction from a thesis administered by first author as a MSc student in department of Horticulture of Islamic Azad University of Sistan and Baluchestan, Zahedan(Science and Research Branch).

REFERENCES

[1]George E, Plant tissue culture procedure-Background Plant propagation by tissue culture, 3rd ed, Springer, Dordrecht, **2008**; pp.1-28
[2]Gutierrez J, Barry-Ryan C and Bourke P, I*nternational Journal of Food Microbiology*, **2008**, 124(1): 91-97
[3]Kenawy E and Abdol-fattah Y,. *Macromolecular Bioscience*, **2002,** 2(6): 261-266
[4]Leifert C, Camotta H and Waites W, *Plant cell tissue and organ culture*, **1992**, 9(2): 153-160
[5]Leifert C, Waites W and Nicholas J, *Journal of Applied Microbiology*, **1989**, 67(4): 353-361
[6] Maskouki M, Mortazavi S and Rad S, *Journal of Agriculture Science and Natural Resoures*, **2005**, 113: 61-69
[7]Moshefi M, Mansouri S and Sharififar F, *Journal of Kerman Universityof Medical Sciences*, **2007**, 14(1): 33-43
[8]Sefidkan F, Sadeghzadeh L and Olia P, *Pajouhesh and Sazandegi***, 2007**, 71: 52-56 (In Farsi with English Summary)