



The Study of Ultrasonic Waves on dormancy-breaking in *Ferula assa-foetida*, *Ferula gummusa* and *Myrtus communis*

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ABSTRACT

Medical plants are adapted to different kinds of dormancy to survive in the worst situations. Regarding to ecological viewpoint, this kind of adaptation is very important in respect to restriction of genetic draft and decrease of genetic resource of medicinal plants. This leads to plant survival even after prolonged drought. Since some of them are endangered species and in the other hand increasing in the production and culture of medicinal plants this turns to a problem. To overcome this problem scientists apply different kinds of treatments (like heat, chemical and physical treatments). So to overcome the germination problem of *F. assa-foetida*, *F. gummusa* and *Myrtus communis*, four different times of 42 KHz ultrasonic waves (0, 4, 8 and 12 minutes) were applied on the seeds of this plant. The experiment was conducted based on completely randomized block design with four replications. The collected data were evaluated by SPSS 14. The essential oil of seeds was obtained by hydrodistillation. Oil of the best treatment of *F. gummusa* was analyzed by GC and GC/MS. In the current study we reported the existence of *F. assa-foetida* in Oshtorankoh mountain for first time. Treatment Results showed that: There were significant differences between ultrasonic waves' treatments (4, 8 and 12 minutes) and control (0 min). 30 days after treatments, the percentages of seed germination for each treatment of *F. assa-foetida* were 35, 75, 57/5 and 62/5%, for *F. gummusa* were 25, 60, 52/5, 45 and for *Myrtus communis* were 0, 42.5, 50, 35 in 4, 8 and 12 minutes ultrasonic waves' treatments and control (0 min) respectively. In this study, 4 minute ultrasonic wave's treatment clearly provides the best results for all species therefore ultrasonic waves method is a cost effective way for increasing seed germination rate in *F. assa-foetida*, *F. gummusa* and *Myrtus communis*. GC and GC/MS results showed differences in the composition of the essential oil of control and 4 minute treatment in *F. gummusa*.

Keywords: Ultrasonic waves, germination, *Ferula assa-foetida*, *Ferula gummusa*

INTRODUCTION

The family Umbelliferae (Apiaceae) includes 300 genera and 3000 species. Genus *Ferula* has 140 species widely spread from the Mediterranean region to Central Asia (Rahman *et al.* 2005). The seeds of these plants like many other medical plants have a long dormancy. The dormant seeds do not germinate on the mother plant hence there is enough time for dispersal of seeds which is a very useful feature. While most garden plant seeds lose their dormancy before or shortly after they are separated from the mother plant,

most wild plants assume a long dormancy (Otrosky *et al.* 2009). In line with the survival of the fittest rule medical plants have adapted diverse kinds of dormancy to survive in the worst situation. Regarding to ecological viewpoint, this kind of adaptation is very important in respect to restriction of genetic draft and decrease of genetic resource of medicinal plants. This leads to plant survival even after long drought. But because of increase in the production and culture of medicinal plants this turns to a problem. To overcome this problem scientists apply different kinds of treatments (like heat, chemical and physical treatments). Nowadays ultrasonic waves are wholesale used in the medicine, biotechnology, physics, chemical and mechanical engineering. Ultrasonic effects exerts by cavitation. Cavitation is the formation and –sometimes- the implosion of microbubbles in liquid undergone ultrasonic waves. The structure and function of biomolecules may be changed by ultrasonic waves (Yaldagard M. *et al.* 2008). In the present research we tried to use this time effective new method, ultrasonic waves, to overcome the problem.

MATERIALS AND METHODS

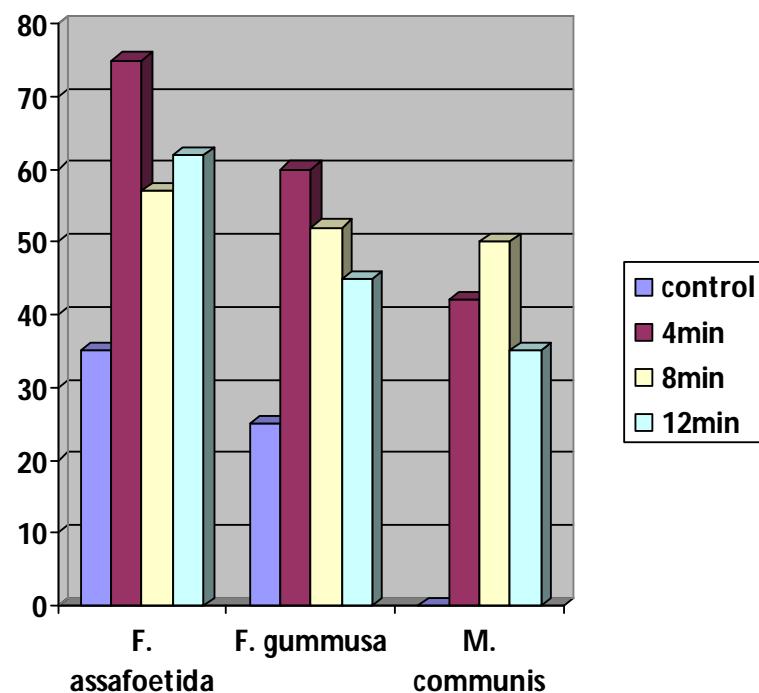
As we reported in the current study for first time we identified and collected seed of *F. assa-foetida* from Oshtorankoh Mountains and also prepared *F. gummusa* from Pakan Seed company. Then the seeds of this plant were transmitted to lab. After inspection of embryos with tetrazolium test. To sterilize the seeds were placed in 2/5% sodium hypochlorite for 20 minute. To overcome the germination problem, four different times of 42 KHz ultrasonic waves (0, 4, 8 and 12minutes) which generated by Digital Ultrasonic CD-4820 were applied on the seeds of this plant. The experiment was conducted based on completely randomized block design with four replications (30 seeds for each replication). In order to compare the effect of material of Petri dishes on germination, the seeds were studied in both plastic and glass Petri dishes. For control, no ultrasonic waves (0 min) were treated. The collected data were analyzed by SPSS 14 software and treatment means were compared using the least significant difference (LSD) test at $P < 0.05$. Seeds of control and 4 minute treatment were subjected to hydrodistillation using a Clevenger-type apparatus to produce light yellow oils in 3 hours for each sample. The oil was stored in a sealed glass vial in the dark place at 4 °C. Essential oil of control and the best treatment of *F. gummusa* was analyzed by Analytical GC & GC/MS (Agilent type with HP- 5MS).

RESULTS AND DISCUSSION

The results of variance analysis of data showed that there were significant differences between ultrasonic waves' treatments (4, 8 and 12 minutes) and control (0 min) in all species ($P < 0.05$). The percentages of seed germination are shown in table 1 and chart1. In the current study for first time we identified and collected *F. assa-foetida* from Oshtorankoh Mountains (33°22'27.93 N, 49°11'29.09E, el. 2000)

Table 1- The percentages of seed germination

Species	<i>Myrtus communis</i>	<i>Ferula foetida</i>	<i>assa-</i>	<i>Ferula gummusa</i>	
Treatments					Glass Petridish Plastic Petridish
0 min	0	35		25	15
4min	42.5	75		60	37/5
8min	50	57/5		52/5	40
12min	35	62/5		45	42/5
Chilling 4°C/2 month	0	Not did		Not did	Not did
Chilling 4°C/1 month	0	Not did		Not did	Not did

**Chart 1:** present of germination

GC and GC/MS results showed that the major components of the oil were α -pinene • β - pinene • Cymene • σ - 3- carene • Limonene • Terpinolene • Fenchyl acetate • Carvacrol methyl ether in different percentage. Sabinene was found just in the essential oil of control. As it is clear in table 1, using glass Petri dishes is more effective on germination than plastic ones. Vice versa we expected rate of fungal contamination in plastic Petri dish was more than glass one. So both fungal contamination and the effect of plastic on light waves might be caused such differences in results. Although ultrasonic waves show positive impact on percentage of germination, germination in 8 and 12 minute treatments are less than 4 minute in both species. It can be because of generation of some kind of stresses in the seeds after so long ultrasonic treatment (8 and 12). However some studies (Otrosky *et al.*, 2009 and Rajabian *et al.*, 2007) showed 90% germination rate in *Ferula assa-foetida* but their methods not only waste long time (28 days to 12 weeks) but also need expensive hormones while by using ultrasonic waves we manage to save time and money. Findings of Yaldagard M. *et al.* (2008) show that alpha-amylase activity was increased as a result of the increasing germination rate in the seed treated by ultrasonic. Coincide with Yaldagard M. *et al.* (2008) "Shell fragmentation dramatically decreases the resistance of the seeds shell against the water diffusion and facilitates the passage of the water molecules across the cell wall. Therefore as a result of increasing the mass transfer rate of the target components, the sonicated tissue absorbs an extra volume of water so that the absorbed water is given to embryo most freely and simply. This could be inducing the more alpha-amylase release and lead to quicker germination and faster embryo growth, such that pretreatment with ultrasonic waves resulted in better germination after sonication." Besides of Yaldagard M. *et al.* (2008) opinion, we clearly observed fragmentation or enlargement in the pores size in the *Lavandula* seed's shell in our previous study (Saki *et al.* 2010) on ultrasonic effects on *Lavandula vera* germination. After sonication, it was clearly proved to us by exiting jelly materials from *Lavandula vera* seeds instantly after using ultrasonic waves in compare to untreated seeds. Fragmentation or enlargement in the pores size was clear in *Lavandula vera* by existence of inhibitor jelly materials around the embryo preventing germination. But after sonication these jelly goes out and the water is easily absorbed and conveyed to the embryo. In this study, 4 minute ultrasonic wave's treatment clearly provides the best results for both *F. assa-foetida* and *F. gummusa* species, Although in *Myrtus communis* the best results was prepared by 8 min treatment, respecting to a little difference between 4 and 8 min treatments in economically view the best result was 4min treatment too. In conclusion, ultrasonic wave's method is a cost effective way for increasing seed germination rate in *Ferula assa-foetida*, *Ferula gummusa* and *Myrtus communis*.

REFERENCES

- Khosravi H. and Mehrabi A. (2006). Economic study of Ferula harvesting in Tabass region. Iranian J. Natural Res. 58(4):933-943.
- Nasiri Seyed Mehdi and Abdali Nargess. (2011). The first report of *Ferula assa-foetida* in Oshtorankoh (Doroud- Lorestan-Iran). The 2nd national biology congress of researchers, Tehran, Iran.
- Otrosky M., Zamani A., Khodambashi M., Ebrahimi M. and Struik P.C. (2009). Effect of exogenous Hormones and chilling on dormancy breaking of seeds of Asafoetida (*Frulla assafoetida* L.). Research Journal of Seed Science. 2(1):9-15.
- Rahman M.U. and Gul S. (2005). Antimicrobial activities of Ferula assafoetida oil part I. Inhibition of a sexual reproduction stages of food borne molds. Jour.Chem.Soc.Pak. 27(4):423-428.

Rajabian T., Saboora A., Hassani B. and Fallah Hosseini H. (2007). Effects of GA₃ and chilling on seed germination of *Ferula assa-foetida*, as a medicinal plant. Iranian Journal of Medicinal and Aromatic Plants. 23(3): 391-404.

Saki T, Nasiri S.M., Abdali N. and Aliniaiefard. (2010). The study of ultrasonic waves on seed germination in *Lavandula vera*. Scientific Conference on Medicinal Plants Industry Development in Iran, Tehran-Iran.

Yaldagard Maryam, Mortazavi Seyed Ali and Tabatabaie Farideh. (2008). Influence of ultrasonic stimulation on the germination of barley seed and its alpha-amylase activity. African Journal of Biotechnology. 7(14): 2465-2471.