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# Effect of Plant Essential Oils on Arachidonic Acid and Oil Production by *Mortierella alpine* CBS 754.68

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**The effects of exogenous plant essential oils and methanolic extracts on Mortierella alpine and the profile of fatty acids were investigated. The results showed that the plant's essential oils and methanolic extracted oils strongly impacted on the amount of biomass, oil and AA production by Mortierella alpine. The highest and lowest positive effect on increasing the biomass relative to the control was reported from *F. assa-foetida* and *L. stoechas*. The results also showed that the most positive impact of increasing the oil and arachidonic acid level are related to R. *officinalis* and *F. vulgare*.**

**Kay Words**: *Mortierella alpine;* Arachidonic acid, Oil, Mycelia inhibition, Plant's essential oils.

The use of fungi for the production of commercial products is ancient, but it has increased rapidly over the last 50 years. Microbial lipophilic compounds, called single cell oils (SCO), present a potential industrial and financial interest due to their specific characteristics[1, 2, 3]. Especially, various oleaginous Zygomycetes have been used in order to produce lipids rich in polyunsaturated fatty acids (PUFA) of medical and dietetical interest, such as Arachidonic acid (AA). Some oleaginous fungi produce triacylglycerides enriched with fatty acids of pharmaceutical relevance. Within the genus *Mortierella*, *M. alpina* is a well-known producer of arachidonic acid[4].

AA, a long chain polyunsaturated fatty acid (PUFA) of the omega-6 class (5, 8, 11, 14-eicosatetraenoic acid), plays important roles in the structure and function of biological membranes. Many expert organizations including FAO/WHO recommend using the supplements of AA in the infant formula [5]. An extensive research on the production of PUFA by the fungus *Mortierella* in submerged culture was carried out over the past several years.

In the case of *Mortierella* genus, it was shown that the composition of the growth media plays crucial roles in mycelia growth and the fatty acid composition of the mould body [6- 16].

In the present communication, the effects exogenous plant essential oils and methanolic extracts on Mortierella alpine, oil and the profile of fatty acids were investigated and discussed.

## METHODS

### Microorganism, inoculums preparation and culture conditions

*Mortierella alpina* CBS 754.68 was purchased from Centraalbureau Schimmelcultures (CBS, Netherlands). The fungus was maintained in malt agar and sub cultured every two months. It was stored at 3±1°C. *M. alpina* CBS 754.68 was initially grown on sterile malt agar in petri dishes at 22°C for 7 days and then transferred to the seed culture medium. The seed culture medium contained (g/L): glucose 30 and yeast extract 7.

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The seed culture of 100 ml was incubated at 25°C for 2 days in a shaker at a rotation spin of 180 RPM. Then the content of the seed culture flask was mixed well by a miller to produce mycelium suspension. All the fermentation experiments were performed in 500 ml Erlenmeyer flasks with 100 ml of the fermentation medium.

The fermentation medium in each flask was inoculated with 5% (v/v) of mycelium suspension of the seed culture. All of the experiments were carried out in two replications.

**Lipid extraction**

Fungal mycelia were harvested by suction filtration, washed with 50 mL distill water, dried at 105°C for 2 h, and weighted to obtain the dry cell weight (DCW)[17]. The dried mycelia were ground into a fine powder to be subsequently extracted with an organic solvent. The lipid content of the grounded powder was extracted with 2 mL

N-hexagon through an ultrasonicator with a centrifugation rate 2000 RPM for 3 h, thereafter[18, 19]. The oil biomass was determined by soxhlet apparatus.

**Fatty acid analysis**

The extracted oil was dissolved in 5 mL of NaOH 2% in methanol solution and methylated by 2.175 mL of $BF_3$. The methylated fatty acids were separated from the aqueous layer through adding a saturated NaCl and dissolving in n-hexane[20]. The PUFA content was determined by a gas chromatography UNICAM 4600 (United Kingdom) equipped with a capillary BPX70 column (30 m × 0.25 mm i.d., 0.25 mm film thickness; SGE, USA) and flame ionization detector. Nitrogen was used as a carrier gas under the pressure of 20 kPa. The injector and detector temperatures were maintained at 250°C and 300°C, respectively. The oven was maintained at 160°C for 6 minutes, then increased to 180°C at the 20°C / min, maintained at 180°C for 9 minutes, increased further to 190°C at the 20°C / min and finally maintained at 190°C for 25 minutes. The injection volume was 0.2 µL, with a split ratio of 50:1. The fatty acids were identified and quantified using the methyl esters of the quantitative standard fatty acids supplied by Sigma (St. Louis, USA). Pentadecanoic acid (15:0) was used as the internal standard.

**Determination of the *fungistatic and fungicidal* activity of the plant's essential oils and methanolic extracted oils on *Mortierella alpina***

The essential oils of *Zataria multiflora* Boiss., *Ferula gummosa Boiss., Eucalyptus camaldulennsis* Dehn., *Thymus kotschyanus* Boiss. & Hohen., *Thymus vulgaris* L., *Mentha longifolia* L., *Salvia mirzayani* Rech., *Zhumeria majdae* Rech.f. & Wendelbo., *Cuminum cyminum* L., *Mentha spicata* L., *Foeniculum vulgare Mill., Lavandula stoechas L., Rosmarinus officinalis* L., *Ferula assa-foetida* L. and methanolic extracts of *Ziziphus spina-christi* (L.) Desf., *Urtica dioica* L. and *Matricaria recutica L.* Used in this study was supplied from Barij Essence Co. of Kashan and the departments of plant pathology and entomology of Tarbiat Modares University (TMU). The essential oils and methanolic extracts were sterilized using 0.2µm filters (Orange Scientific, Belgium). The antifungal assay was carried out *in vitro*, in petri dishes (5cm in diameter) containing potato dextrose agar (PDA). As the temperature of the medium (PDA) reached to about 40°C, specific concentration (0 and 200 µL/L) of methanolic extracts and 50% stocks of the plant's essential oils (diluted in ethanol 96%) were added to PDA and mixed thoroughly. The rate of mycelia inhibition was measured after placing an active mycelia plug of fungus on petri dishes containing PDA with specific concentrations of essential oils and methanolic extracts and then incubated at 28±°C.

**The observations were recorded on the seventh day and the percentage of mycelia inhibition was calculated by the following formula**

$$\text{Percentage of mycelia inhibition} = d_c - d_t / d_c \times 100$$

where $d_c$ is the mean colony diameter of the control sets and $d_t$ is the mean colony diameter of the treatment sets[21]. The experiments were conducted in a completely randomized design with two concentration rates and two replications.

## RESULTS

**The Inhibitory effects of exogenous plant essential oils and methanolic extracts on Mortierella alpine**

The results indicated the mycelia inhibitions of plant essential oils of Z. *multiflora, F. gummosa, E. camaldulennsis, T. kotschyanus, T. vulgaris , M. longifolia, S.mirzayani, Z. majdae* and methanolic extracts of *Z. spina-christi*,

**Table 1.** Mycelia inhibition of plant essential oils and methanolic extracts on Mortierella alpine.

| Plant | Family | Percentage of mycelia inhibition |
|---|---|---|
| *Z. multiflora* | Lamiaceae | 81.16±1 |
| *C. cyminum* | Apiaceae | -8.82±0.9 |
| *M. spicata* | Lamiaceae | -5.22±1 |
| *F. vulgare* | Apiaceae | -2.80±0.7 |
| *F. gummosa* | Apiaceae | 10.25±2 |
| *L. stoechas* | Lamiaceae | -4.81±0.7 |
| *Z. majdae* | Lamiaceae | 3.07±1 |
| *Z. spina-christi.* | Rhamnaceae | 2.33±0.5 |
| *U. dioica* | Urticaceae | 2.89±0.7 |
| *R. officinalis* | Lamiaceae | 1.64±0.6 |
| *E. camaldulensis* | Myrtaceae | 4.15±1 |
| *T. kotschyanus.* | Lamiaceae | 96.07±1.5 |
| *T. vulgaris* | Lamiaceae | 64.66±1.7 |
| *M. longifolia.* | Lamiaceae | 35.24±2 |
| *S. mirzayani* | Lamiaceae | 7.66±0.7 |
| *F. assa-foetida* | Apiaceae | -1.15±0.4 |
| *M. recutica* | Asteraceae | 3.61±0.3 |

*U. dioica* and *M. recutica* and *R. officinalis* are positive but those of *C. cyminum*, *M. spicata* , *F. vulgare*, *L. stoechas*, and *F. assa-foetida* are negative (Table 1). The negative inhibition of mycelia means these essential oils did not cause the fungal growth to be decreased but increased its growth rate as compared with the control treatment. Considering the mentioned results, a concentration rate 200 ppm for each essential oil (with negative mycelia inhibition) was used in the submerged medium and their impact on the oil and AA levels was then evaluated.

**The Effect of essential oils on profile of fatty acids**

All the treatment expects *F. vulgare* showed negative effects on the production of AA. besides, R. officinalis and L. stoechas caused the oil production to be increased while the other essential oils showed a negative effect on the oil production (Table 2). The results also showed that all the essential oils except *R. officinalis* had a positive effect on the amount of biomass. The highest and lowest positive effect on increasing the biomass relative to the control was reported from *F. assa-foetida* (16.76%) and *L. stoechas* (1.2%) respectively.

**Table 2.** Effect of plant essential oils on biomass, oil production and profile of fatty acids in comparison with control (soybean 10 g/L, glucose 40 g/L, temperature 17°C).

| Treatment | Cell dry Wight (%) | OIL(%) | C16:0 palmitic acid | C18:0 stearic acid | C18:1 Oleic acid | C18:2 linoleic acid | C18:3 linolenic acid | AA(%) | EPA (%) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 1.9±0.04 | 35±1 | 22±0.8 | 7.6±0.7 | 12±1 | 9.6±0.9 | 2.9±0.9 | 42±1 | 3.3±1 |
| *F. assa-foetida* | 2.27±0.03 | 24±1.3 | 17.6±0.7 | 7±0.8 | 13±1.1 | 9.7±1 | 2.5±1 | 37±0.8 | 5±1.2 |
| *M. spicata* | 2.04±0.05 | 27±0.9 | 24±1 | 9.2±1 | 16±0.9 | 11.5±0.7 | 1±0.8 | 29±1.1 | 3±0.7 |
| *F. vulgare* | 2±0.06 | 27±0.8 | 15±0.9 | 7±1.2 | 11±0.8 | 10±0.8 | 1±0.7 | 45±0.9 | 4.7±0.8 |
| *C. cyminum* | 2.2±0.03 | 31±1.2 | 18±1.1 | 7.7±0.9 | 14±1.1 | 9.7±1.1 | 2±1 | 37±1.2 | 5±1 |
| *R. officinalis* | 1.81±0.05 | 47±1.1 | 13±0.6 | 9.6±0.8 | 8.8±0.6 | 10±1.2 | 2±1.3 | 41.5±0.7 | 3±1.2 |
| *L. stoechas* | 1.99±0.04 | 42±0.7 | 23±1 | 11±1 | 17±1.1 | 13±0.9 | 2±0.7 | 23±0.6 | 2±1.1 |

## CONCLUSION

The plant essential oils and methanolic extracted oils potentially impacted on the amount of biomass, oil and AA production by *Mortierella alpine*. Only *F. vulgare* increased the AA as compared with the sample control. *L. stoechas* and *R. officinalis* potentially increased the oil production as compared with the sample control. All of the treatments expect *R. officinalis* caused biomass increase. Nisha & Venkateswaran (2008)[22] reported that media supporting high biomass accumulation was not concomitant with their ability to produce PUFA including AA while our Results showed that increase of biomass accumulation caused oil production decrease in biomass. In such away, the treatment that the most positive impact of increasing the oil is related to *R. officinalis* that having a negative effect on the growth of Mortirella fungus". However, production of AA had different producer. All the treatment expects *F. vulgare* had

a negative effect on production of AA. According to the results by Shimizu *et al*., 1991[23], it is inferred that decreasing in AA is caused by decreasing or suppression of some unsaturated gene related to AA biosynthesis. Results also showed that lipid biosynthesis is different from AA biosynthesis.

## REFERENCES


1. Ratledge C Fatty acid biosynthesis in microorganisms being used for Single Cell Oil production.*Biochimie*, 2004; **86**: 807-15.
2. Ratledge C Single cell oils--have they a biotechnological future? *Trends Biotechnol*. 1993; **11**: 278-84.
3. Certik M, Shimizu S Biosynthesis and regulation of microbial polyunsaturated atty acid production. *J Biosci Bioen*. 1999; **87**: 1-4.
4. Totani N, Oba K. The filamentous fungus Mortierella alpina, high in arachidonic acid. *Lipids*. 1987; **22**: 1060-1062.
5. Yamada H, Shimizu S, Shinmen Y, Kawashima H, Akimoto K. Biotechnological processes for production of polyunsaturated fatty acid, *J Disper Sci Technol*. 1989; **10**: 561-579.
6. Bajpai P, Bajpai PK, Ward OP. Eicosapentaenoic acid (EPA) formation: comparative studies with Mortierella strains and production by Mortierella elongate. *Mycol Res*, 1991; **95**: 1294-1298.
7. Eroshin VK, Dedyukhina EG, Chistyakova TI, Zhelifonova VP, Kurtzman CP, Bothast RJ. Arachidonic-acid production by species of Mortierella. World *J Microbiol Biotechnol*, 1996; **12**: 91-96.
8. Funtikova NS. Mysyakina ISk. Synthesis of gammalinolenic acid by mucaraceous fungi utilizing exogenous fatty acids. *Microbiol*, 1997; **66**: 76-79.
9. Hansson L, Dostalek M. Effect of culture conditions on mycelial growth and production of gamma-linolenic acid by the fungus Mortierella ramanniana. *Appl Microbiol Biotechnol*, 1988; **28**: 240-246.
10. Jareonkitmongkol S, Kawashima H, Shirasaka N, Shimizu S, Yamada H. Production of dihomo-gamma-linolenic acid by a delta5-desaturase-defective mutant of Mortierella alpina 1S-4. *Appl Environ Microbiol*, 1992; **58**: 2196-2200.
11. Jareonkitmongkol S, Sakuradani E, Shimizu S. A novel delta5-desaturase-defective mutant of Mortierella alpina 1S-4 and its dihomo-gamma-linolenic acid productivity. *Appl Environ Microbiol*, 1993; **59**: 4300-4304.
12. Jareonkitmongkol S, Shimizu S, Yamada H. Fatty acid desaturation-defective mutants of an arachidonic-acid-producing fungus, Mortierella alpina 1S-4. *J Gener Microbiol*, 1992; **138**: 997-1002.
13. Kavadia A, Komaitis M, Chevalot I, Blanchard F, Marc I, Aggelis G. Lipid and gamma-linolenic acid accumulation in strains of Zygomycetes growing on glucose. *J Am Oil Chem Soc*, 2001; **78**: 341-346.
14. Kawashima H, Akimoto K, Higashiyami K, Fujikawa S, Shimizu S. Industrial production of dihomo-gammalinolenic acid by a delta5-desaturase-defective mutant of Mortierella alpina 1S-4 fungus. *J Am Oil Chem Soc*, 2000; **77**: 1135-1138.
15. Kendrick A, Ratledge C. Lipids of selected molds grown for production of n _ 3 and n _ 6 polyunsaturated fatty acids. *Lipids*, 1992; **27**: 15-20.
16. Kendrick A, Ratledge C. Cessation of polyunsaturated fatty acid formation in four selected filamentous fungi when grown on plant oils. *J Am Oil Chem Soc*, 1996; **73**: 431-435.
17. Koike Y, Cai HJ, Higashiyama K, Fujikawa S, Park EY. Effect of consumed carbon to nitrogen ratio on mycelia morphology and arachidonic acid production in cultures of Mortierella alpine. *J Biosci Bioeng*, 2001; **91**: 382-389.
18. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem*, 1957; **226**: 497-509.
19. Jang HD, Lin YY, Yang SS. Effect of culture media and condition on polyunsaturated fatty acid production by Mortierella alpine. *Bioresource Technol*, 2005; **96**: 1633-1644.
20. Metcalfe LD, Schmitz AA, Pelka JR. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal Chem*. 1996; **38**: 514-515.
21. Tripathi P, Dubey NK, Shukla AK. Use of some essential oils as post-harvest botanical fungicides in the management of grey mould of grapes caused by Botrytis cinerea. World *J Microbiol Biotechnol*, 2008; **24**: 39-46.
22. Nisha A, G. Venkateswaran. Effect of Culture Variables on Mycelial Arachidonic acid Production by Mortierella alpine. *Food and Bioprocess Technology*, 2008; **4**: 232-240, DOI: 10.1007/s11947-008-0146-y.
23. Shimizu S, Akimoto K, Shinmen Y, Kawashima H, Sugano H, Yamada H, Sesamin is a potent inhibitor of del 5 desaturase in polyunsaturated fatty acid biosynthesis. *Lipids*, 1991; **26**(7): 512-516.