# *In vitro* effect of peppermint (*Mentha* x *piperita* L. var. *officinalis*) water extracts on *Fusarium* fungi

ELŻBIETA BOLIGŁOWA[1*], ELŻBIETA PISULEWSKA[2], KATARZYNA GLEŃ[1]

[1]Department of Agricultural Environment Protection
[2]Department of Crop Production,
Agricultural University,
al. Mickiewicza 21,
31-120 Kraków, Poland
[*]corresponding author: rrboligl@cyf-kr.edu.pl

S u m m a r y

Laboratory analyses were conducted to compare the effect of two doses of morphologically diversified English and French peppermint (*Mentha* x *piperita* L. var. *officinalis*) water extracts on linear growth, biomass and sporulation of selected *Fusarium* fungi. Obtained results revealed the strongest inhibitory effect of peppermint extracts only upon *Fusarium sulphureum*. In other analysed fungi (*F. culmorum, F. graminearum and F. avenaceum*) no obvious fungistatic activity of tested water extracts or their doses was registered.

*Key words: water extract, peppermint, Fusarium fungi*

Harmful effect of synthetic pesticides on the environment necessitated seeking natural plant protection means based on microorganisms and compounds isolated from plants [1-3]. Application of preparations based on natural compounds makes possible eliminating or diminishing use of chemicals for plant protection, improvement in the quality of raw material for organic food production, protection of the natural environment through their weakened influence and their easier biodegradation [4]. At present there are few plant preparations registered and attempts have been made to enlarge a range of these products. Except ready made preparations, also plant water extracts may be used for plant protection against agrophages. A number of laboratory tests revealed inhibitory effect of plant sap [5], extract [6] or water extract [7-9] upon some phytopathogenic fungi. Various plant species may be a potential source of natural compounds showing fungicidal properties [10]. The compounds of antibacterial and antifungal character are

glycosides, alkaloids, phenols, saponins and ether oils [11, 12]. According to Łakota et al. [12], quantity and quality of these compounds in plants depend on many factors: species and growth phase of plant, weather and soil conditions.

Some authors think [13] that extracts from oil plants reveal fungistatic properties. Peppermint (*Mentha* x *piperita* L. var. *officinalis*) may be counted among these plants. Black English peppermint is cultivated for menthol oil and white French peppermint mainly for tea production. In the opinion of Ożarowski and Jaroniowski [14], the main component of peppermint is ether oil in which menthol is dominant. Apart from that mint raw material contains also tannins, flavonoids, phenol acids and mineral salts. A bacteriocidal effect is ascribed to mint decoctions and extracts.

The hitherto available literature reveals inhibitory effect of some plant water extracts towards some plant pathogens [5, 6, 9, 15, 16]. However, there are no reports on the influence of extract of morphologically diversified English and French peppermint (*Mentha* x *piperita* L. var. o*fficinalis*) on linear growth, biomass and sporulation of selected fungi from *Fusarium* genus.

## MATERIALS AND METHODS

Leaves of English peppermint (black mint) and French (white mint) from strict field experiments conducted in 2006 on private farm at Michałów (małopolskie province) were sampled for the analyses. The tillage, fertilization and plant husbandry were conducted according to agrotechnical recommendations.

Water extracts in the form of decoctions were prepared of air-dried mass of mint leaves, according to Suder and Grebber procedure [17] and then, after filtration through bacteriological filters a dose of 25 and 50 μg/ml was added to the PDA medium. The ready media were inoculated with an agar circle, 5 mm in diameter overgrown with two-week old mycelium of *Fusarium culmorum* (W.G.Sm.) Sacc., *F. graminearum* Schwab, *F. sulphureum* Schlecht, *F.avenaceum* (Corda ex Fr.) Sacc. Pathogenic microorganisms tested in the experiment originated from Department of Agricultural Environment Protection own collection. The controls were Petri dishes with medium without water extract of peppermint. The fungi were cultured in a thermostat (at the temperature of 23°C ± 1°C) in five replications.

Since mycelium growth increment appeared, the colony diameter was being measured for seven days, every day. The linear growth of analysed organisms was presented as a difference of fungi growth on control Petri dishes in comparison with dishes containing media with plant water extract supplements. The coefficients of linear growth rate and inhibition according to Abbott were also computed.

Three weeks after the experiment outset conidial sporulation of test fungi was assessed by counting number of macroconidia in Bürker hemocytometer.

Growth of the tested fungi biomass was conducted in 300 ml Erlenmayer flasks on 100 ml of modified medium (without agar) with use of plant water extracts,

similarly to experiment with fungi linear growth. In the inoculation chamber the fungi culturing was conducted for 21 days at room temperature of ca. 22°C. After this period the post-culturing liquid with mycelium was filtered through filtration paper. Subsequently the mycelium was dried on a sterile glass at 80°C until constant weight was reached.

The obtained results were subjected to ANOVA. The significance was determined by t-Student test.

## RESULTS AND DISCUSSION

The obtained research results revealed significant inhibition of tested fungi colony growth rate under the influence of peppermint extract in comparison with control (Table 1). Also other authors [5, 7-9] reported fungi growth inhibition under the influence of plant water extracts. Authors' own investigations showed different sensitivity of fusarium fungi to presence of water extracts. Among the tested fungi organisms, *F. sulphureum* revealed the highest sensitivity, as has been evidenced by the most limited rate and inhibition of its growth (Table 1, Fig. 1). Diversified effect of plant extracts on phytopathogenic fungi growth was confirmed by Burgieł [7], Łakota et al. [12] as weel as Boligłowa and Znój [9]. However, the above mentioned authors analysed extracts from other plants and conducted experiments on different fungi genera (*Fusarium, Alternaria, Botritis*). In our experiment the rate of tested fusarium fungi growth depended not only on fungus species but also on plant of which the extract was prepared, and finally on dose supplied to the PDA medium. Among many biologically active substances in peppermint, fungistatic effect should be ascribed to ether oils. Daferera et al. [13] who applied ether oils of aromatic plants (oregano, thyme and lavender) also found inhibition of growth in *Botritis cinerea* and *Fusarium* sp. Results of the authors' own investigations demonstrated that a supplement of 25 µl/ml of French mint extract to PDA medium led to significant inhibition of *F. culmorum* and *F. sulphureum* colony growth with regard to both analysed concentrations of English mint (Table 1). The obtained results reveal that water extracts prepared from morphologically diversified plants differ from their chemical composition. In the opinion of Daferera et al. [13], activity of isolated oils towards microorganisms is not unanimous and depends on their chemical composition and dose. The research shows that higher dose of French mint water extract had inhibitory effect only upon *F. avenaceum* (Fig. 1).On the other hand, water extract of English and French peppermint did not have any marked effect on the growth rate of *F. graminearum* (Table 1). Analysis of data compiled in Table 2 reveal that French mint had stronger fungistatic properties than the English one.

**Table 1.**

Linear growth coefficients of selected fusarium fungi depending on peppermint extract concentration in medium.

| water extract | dose water extract (µl/ml PDA) | *Fusarium culmorum* | *Fusarium graminearum* | *Fusarium sulphureum* | *Fusarium avenaceum* |
|---|---|---|---|---|---|
| English peppermint | 50 | 64.55 | 51.66 | 29.14 | 35.97 |
| | 25 | 65.80 | 50.55 | 29.09 | 37.99 |
| French peppermint | 50 | 61.01 | 51.98 | 29.04 | 34.28 |
| | 25 | 60.32 | 50.71 | 25.83 | 36.01 |
| control | | 85.60 | 91.00 | 69.24 | 46.15 |
| $LSD_{0.05}$ | | 2.60 | 1.57 | 1.82 | 1.98 |

**Table 2.**

Peppermint effect on inhibition of linear growth rate of selected fusarium fungi.

| water extract | *Fusarium culmorum* | *Fusarium graminearum* | *Fusarium sulphureum* | *Fusarium avenaceum* | mean | $LSD_{0.05}$ |
|---|---|---|---|---|---|---|
| English peppermint | 27.60 | 44.44 | 53.08 | 22.28 | 36.85 | |
| French peppermint | 31.49 | 43.49 | 55.09 | 26.91 | 39.23 | 1.07 |
| mean | 29.50 | 43.96 | 54.08 | 24.60 | | |
| $LSD_{0.05}$ | | | 1.52 | | | |

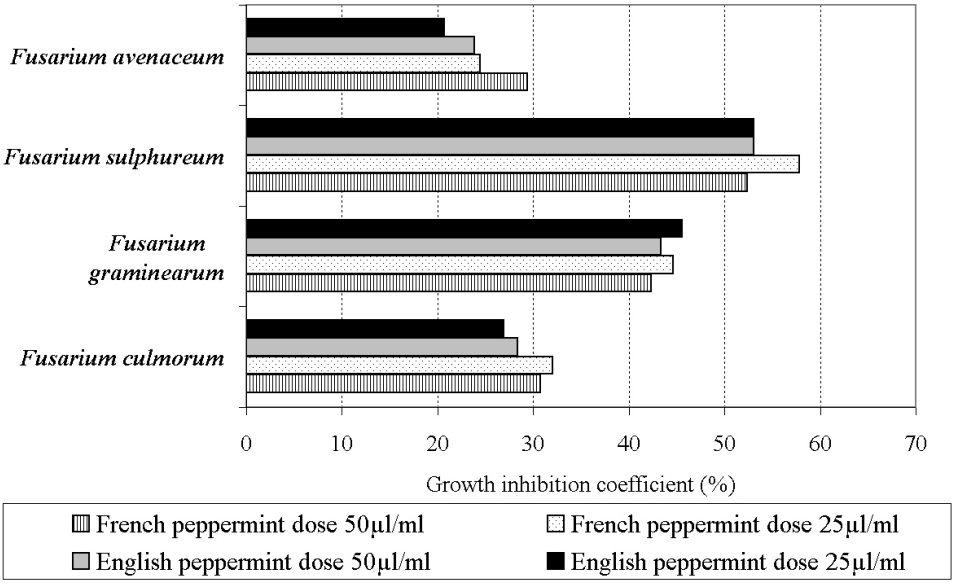


Fig. 1. Inhibition of linear growth of fusarium fungi (%).

Biomass also depended on fungi species, quality of raw material used for extract preparation and its dose (Table 3). The tested extracts significantly diversified the increment of fusarium fungi biomass, except from *F. graminearum*. A lower dose of peppermint (both English and French) supplement to medium stronger inhibited biomass increment in all analysed fungi. The effect was most pronounced for *F.culmorum* (Fig. 2). On the other hand, higher dose (50 µl) of English mint extract stimulated increment of *F. graminearum* and *F. sulphureum* biomass, but especially *F. avenaceum* biomass.

**Table 3.**

Biomass of tested fungi (g).

| water extract | dose water extract (µl/ml PDA) | *Fusarium culmorum* | *Fusarium graminearum* | *Fusarium sulphureum* | *Fusarium avenaceum* |
|---|---|---|---|---|---|
| English | 50 | 0.607 | 0.638 | 0.641 | 0.701 |
| peppermint | 25 | 0.430 | 0.512 | 0.465 | 0.529 |
| French | 50 | 0.649 | 0.553 | 0.509 | 0.595 |
| peppermint | 25 | 0.357 | 0.406 | 0.385 | 0.400 |
| control | | 0.688 | 0.526 | 0.545 | 0.493 |
| $LSD_{0.05}$ | | 0.06 | n. s. | 0.100 | 0.05 |

n. s. – no significant

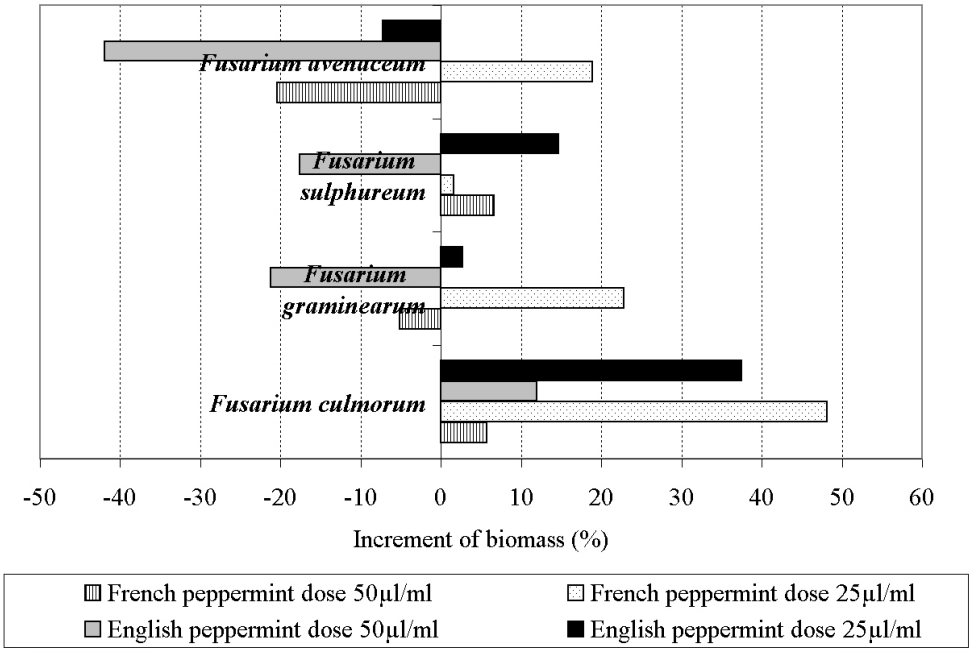


Fig. 2. Inhibition-stimulation coefficient of fusarium fungi biomass increment (%).

Conidial sporulation depended on the fungus species and dose of peppermint water extract applied (Table 4). The extract of English and French peppermint most strongly inhibited sporulation of *F. sulphureum* in comparison with the control. Piotrowski et al. [8] obtained similar results of laboratory tests but for *F. culmorum*, *Botrytis cinerea* and *Alternaria alternata*. Bosshard [15], who tested water extract of common ivy, also registered inhibition of conidial sporulation of *Venturia inaequalis* fungus. In our investigations higher doses of extracts stimulated production of macroconidia only by *F. graminearum*, whereas they limited it in *F. culmorum*. However, in relation to the control more intensive sporulation process in both fungi (*F. culmorum* and *F. graminearum*) was registered under the influence of water extract of English and French peppermint. A similar response of *F.culmorum* to nettle water extract was observed by Burgieł [7].

**Table 4.**

Conidial sporulation of fusarium fungi under the influence of peppermint water extract.

| water extract | dose water extract (µl/ml PDA) | number of spores per 1 $cm^3$ x $10^7$ | | | |
|---|---|---|---|---|---|
| | | *Fusarium culmorum* | *Fusarium graminearum* | *Fusarium sulphureum* | *Fusarium avenaceum* |
| English peppermint | 50 | 32.72 | 33.00 | 4.20 | 4.60 |
| | 25 | 112.10 | 21.23 | 2.75 | 5.11 |
| French peppermint | 50 | 45.00 | 71.08 | 3.68 | 4.20 |
| | 25 | 92,12 | 30.14 | 3.25 | 3.94 |
| control | | 31.14 | 7.54 | 32.20 | 5.27 |

Diversified effect of English and French peppermint extracts phenomenon is probably connected with specifics of chemical compounds composing the plant material, their complex effect and sensitivity of phytopathogen itself, as has been reported by Burgieł and Klein [5].

## CONCLUSIONS

1. The strongest inhibitory effect of water extract of peppermint (*Mentha* x *piperita* L. var. *Officinalis*) was observed only for *Fusarium sulphureum*.
2. No unanimous fungistatic effect of tested water extracts or doses of morphologically diversified English and French peppermint were observed in relation to *F.culmorum*, *F. graminearum* or *F. avenaceum*.

## REFERENCES

1. Pięta D, Machowicz-Stefaniak Z, Wolski T, Kawka S. Preparat furanokumarynowy i próby jego zastosowania jako środka ochrony przed fitopatogenami. Pestycydy 1995; (3):29-36.
2. Orlikowski L B, Skrzypczak Cz, Wojdyła A, Jaworska-Marosz A. Wyciągi roślinne i mikroorganizmy w ochronie roślin przed chorobami. Zesz Nauk AR Kraków 2002; Ses Nauk 387 (82):19-32.
3. Lipa J. Ochrona roślin w rolnictwie ekologicznym – potrzeby i możliwości. Prog Plant Prot/Post Ochr Rośl 2003; 43(1):231-41.

4. Lipa J. Coraz szerszy zakres stosowania biologicznej metody ochrony roślin w Polsce. Ochrona Roślin 2002; (7):10-11.
5. Burgieł Z J, Klein M. Fungistatyczna aktywność wodnych wyciągów i działanie mitotyczne soku z korzeni chrzanu. Zesz Nauk AR Kraków1998; Ses Nauk 57 (333):805-8.
6. Wolski T, Gliński J, Buczek K, Wolska A. Otrzymywanie i charakterystyka roślinnych ekstraktów furanokumarynowych o działaniu przeciwgrzybiczym. Herba Pol 1996; 3 (47):168-73.
7. Burgieł Z. Fungistatyczna aktywność wodnych wyciągów z ziela pokrzywy zwyczajnej (*Urtica dioica*) i korzeni żywokostu lekarskiego (*Symphytium officinale*. Pestycydy 1995; (4):21-5.
8. Piotrowski W, Sas-Piotrowska B, Wyrostkiewicz K, Czajkowski P. Wpływ wyciągów roślinnych na kiełkowanie zarodników niektórych grzybów patogenicznych dla roślin. Zesz Nauk ART Bydgoszcz 1995; Rol 36 (190):139-45.
9. Boligłowa E, Znój K. The effect of plant preparations on growth of selected phytopathogenic fungi. J Res Applic Agricult Engineering 2003; 48 (3):24-7.
10. Malinowski H, Woreta D. Możliwości stosowania naturalnych pestycydów z miodli indyjskiej (*Azadirachta indica* A. Juss) i innych roślin z rodziny *Meliaceae* w ochronie roślin ze szczególnym uwzględnieniem ochrony lasu. Prog Plant Prot/Post Ochr Rośl 1997; 37 (1):131-6.
11. Anioł-Kwiatkowska J, Kwiatkowski S, Berdowski W. Rośliny lecznicze. Atlas. Arkady 1993: 15-16.
12. Łakota S, Kwiatkowski M, Czerwiński Z. Możliwości wykorzystania związków pochodzenia roślinnego do zwalczania szkodników i patogenów roślin. Pestycydy 1993; (1):29-33.
13. Daferera D J, Ziogas B N, Polissiou M G. The effectiveness of plant essential oils on the growth of *Botrytis cinerea, Fusarium* sp. and *Claviobacter michiganensis* subsp. *michiganensis*. Crop Protection 2003; V 22, Issue 1, 39-44.
14. Ożarowski A, Jaroniowski W. Rośliny lecznicze i ich praktyczne zastosowanie. IWZZ 1987: 88-364.
15. Suter H, Graber C. Biologiczna ochrona roślin. MULTICO 1995:23-4.
16. Burgieł Z J, Maliszewska E. Badania nad możliwością wykorzystania wyciągów roślin w ochronie roślin przed fitopatogenicznymi grzybami. Mat Konf 50 Zjazdu PTB - Kraków 1995:115-19.
17. Bosshard E. Effect of Ivy (*Hedear helix*) extract against apple *Scab*. Mildew. Acta Phytopathologica et Entomologia Hungarica 1992; 27,(1-4):135-40.

## ODDZIAŁYWANIE WODNEGO WYCIĄGU MIĘTY PIEPRZOWEJ (*MENTHA* X *PIPERITA* L. VAR. *OFFICINALIS*) NA GRZYBY Z RODZAJU *FUSARIUM* W WARUNKACH *IN VITRO*

ELŻBIETA BOLIGŁOWA[1*], ELŻBIETA PISULEWSKA[2], KATARZYNA GLEŃ[1]

[1]Katedra Ochrony Środowiska Rolniczego,
[2]Katedra Szczegółowej Uprawy Roślin,
Akademia Rolnicza
Al. Mickiewicza 21, 31-120 Kraków
[*]autor, do którego należy kierować korespondencję: rrboligl@cyf-kr.edu.pl

S t r e s z c z e n i e

Badania laboratoryjne dotyczyły porównania oddziaływania dwóch dawek wodnych wyciągów mięty pieprzowej (*Mentha* x *piperita* L. var. o*fficinalis)* angielskiej i francuskiej, zróżnicowanej morfologicznie, na wzrost liniowy, biomasę i zarodnikowanie wybranych grzybów z rodzaju *Fusarium*. Uzyskane wyniki wykazały najsilniejsze działanie inhibicyjne wodnego wyciągu mięty pieprzowej jedynie w odniesieniu do *Fusarium sulhpureum*. W przypadku pozostałych analizowanych grzybów (*F. culmorum, F. graminearum i F. avena-*

*ceum)* stwierdzono brak jednoznacznej aktywności fungistatycznej testowanych wodnych wyciągów oraz ich dawek.

*Słowa kluczowe: wodny wyciąg, mięta pieprzowa, grzyby Fusarium*