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ASSESSMENT OF THE ANTIFUNGAL PROPERTIES OF NETTLE EXTRACTS AGAINST *Fusarium proliferatum*

OCENA WŁAŚCIWOŚCI PRZECIWGRZYBOWYCH POKRZYWY ZWYCZAJNEJ WOBEC *Fusarium proliferatum*

Abstract: The aim of conducted research was to determine the antifungal properties of the leaf and nettle rot extracts on the growth of *Fusarium proliferatum.* The antagonistic properties of nettle extracts at 2.5, 5.0, 10, 20 and 40% concentrations were assayed with a dual culture plate method on PDA and Tomato media. The culturing process was conducted at 26ºC for 9 days and the fungal linear growth was measured every 1-2 days. The positive control in the experiment was Topsin M 500 SC whereas negative the distilled water. The fungistatic activity of nettle extracts was determined against the conidia germination rate index, the growth rate index and the rate of mycelial growth inhibition of *Fusarium proliferatum.* Obtained results shows that the nettle rot extracts inhibited the spore germination (from 7.5 to 47.3%) at all tested concentrations and nettle leave extracts (from 5.9 to 54.0%, respectively) towards negative control, while stimulated compared to positive control. The highest inhibition of the linear growth of mycelium has been observed both for nettle rot and leaf extracts on Tomato medium (9.8 and 11.2%, respectively) and on PDA medium (5.5 and 3.2%, respectively) at 40% concentration towards negative control, while stimulated compared to positive control.

Keywords: stinging nettle, antifungal activity, *Fusarium proliferatum*

Fusarium are ubiquitous in soil and can exist as saprophytes or pathogens in plant tissues. They belong to the most frequent pathogens causing disease and losses of agricultural crops around the world. Particularly the *F. oxysporum, F. solani, F. culmorum and F. proliferatum* are causing severe damage to agriculture in pre- and post-harvest. *Fusarium* spp. belong to the most dangerous pathogens of cereals, tomatoes, corn, asparagus. In addition, they have the ability to produce highly toxic to humans and animals with differentiated structure mycotoxins such as trichothecenes, zearalenone, fumonisin [1-4]. The most important method of protecting the plants against phtytopathogens is the use of fungicides. However, many of which are toxic, non-biodegradable and have undesirable effects on other organisms present in the environment. Therefore, there is a need to find alternative agents for the control of pathogenic fungal diseases in plants. There is a good reason to suppose that the secondary metabolites of plants have evolved to protect them from attack by microbial pathogens. So, natural products from plants (*eg* essential oils, extracts) have great potential as novel fungicide sources for controlling phytopathogens in agriculture [5, 6].

Urtica dioica L. belongs to the family Uriticaceae, commonly called as stinging nettle, is a perennial plant growing in temperate and tropical wasteland areas around the world [5, 7]. The different parts used include leaf, root, stem, flower and modified plant organs. Phytochemical studies revealed the presence of many different valuable classes of organic compounds. The compounds which are reported from the nettle are flavonoids, phytosterols, tannins, proteins, polysaccharydes, essential oil, fatty acids, volatile

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compounds, vitamins and minerals in this plant [7-10]. For this reason U. *dioica* has been reported to have various activities like antioxidant, insecticide, antimirobial antiviral. The effect of nettle extracts on microorganism has been studied by a very large number of researches that indicate activity of the nettle extracts against of bacteria (*eg Bacillus* spp., *E. coli*, *S. aureus*, *P. aeruginosa*) [8, 9, 11, 12] and fungi (*Candida albicans*, *Aspergillus flavus*, *Alternaria alternata*, *Alternaria solani*, *Curvularia lunata*, *Botritis cinerea* and *Rhizoctonia solani* [5, 8, 11-14]. The antifungal effects of the phyto-extracts indicate the potential of selected plant species as a source of natural fungicidal material [5, 13].

The main purpose of this study was to evaluate the antifungal properties of the leaf and root extracts of *Urtica dioica* on the growth and spore germination of *Fusarium proliferatum*.

Materials and methods

In this study the fungistatic properties of the nettle extracts at 2.5, 5.0, 10, 20 and 40% concentrations were determined against the conidia germination, the growth rate index and the rate of mycelial growth inhibition of *Fusarium proliferatum.*

Conidial germination. Analysis of effect the nettle extracts on spore germination was performed with the modifying slide germination method. Fungal cultures were cultivated on PDA medium slants at 25 ± 2ºC for 7 days and the well-developed fungal culture was rinsed with 10 cm^3 of sterile water containing 0.05% Tween 80. Mycelium was filtred from sterile gazue and suspension was adjusted to 10^6 conidia/cm^3 in hemocytemetr. Subsequently 30 mm^3 of nettle extracts and 30 mm^3 of fungal spores were transfered on 1 cm^3 sterile, clean glass slide. Control treatments were prepared by substitution of nettle extracts by Topsin M 500 SC (positive control - PC) and distilled water (negative control - NC). The slides were incubated in humid chambers in triplicate at 25 ± 2ºC for 24 hours. After this period the number of germinated and non-germinated conidia were counted under microscope using x200 magnification. Conidia germination was presented as the germination rate index [15].

Mycelium growth. The antagonistic activity of the nettle extracts were assayed *in vitro* tests with a dual culture plate method using two different media: PDA and Tomato media. First, on this media were inoculated with 0.5 cm^3 of the appropriate concentrations of nettle leaf and nettle root extracts. Next, the media were inoculated with fungal mycelial-disks (diameter of 10 mm) obtained from growing cultures of tested *F. proliferatum* strains. The positive control in the experiment was Topsin M 500 SC whereas the negative one the distilled water. The culturing process was conducted at 25 ± 2ºC for 9 days and the fungal linear growth were measured in two directions on each plate every 1-2 days. Each experiment was run in four replicates, where one was represented by one culturing plate with the growth medium and the mycelial disc. The antagonistic activity of this extracts on the growth of *F. proliferatum* was estimated as the growth rate index [16].

The fungistatic properties of the nettle extracts have been designated on the basis of the percentage reduction of the linear growth of the *F. proliferatum.*

Results and discussion

The plants are the source of many important potential bioactive metabolites used in the limiting the growth of microorganisms. Certain plants extracts act in many ways on various fungi and may be applied to the crop as agricultural chemicals. *Urtica dioica* may also be leading factor in wide range of activities against many phytopathogens, where these pathogens have developed resistance against specific fungicides [5, 8]. Therefore, the antifungal activity extracts of nettle leaf and nettle root of *Urtica dioica* on the conidia germination and linear was evaluated toward tested *F. proliferatum.* Conducted studies have shown differences in fungistatic activity of extracts, depending on theirs concentrations and the stage of the fungus. The potential of infectious the pathogenic fungi largely depends on their ability to produce spores. Usually they make the infection of plant organs, and less frequently the perpetrator of the first stage of the disease are hyphae. The extracts of stinging leaf and nettle root showed the capacity to inhibit the spore germination and linear growth of the *F. proliferatum.*

The degree of fungal conidia germination was different, depending on the kind of extracts and also its concentrations. The results are shown in Figure 1.

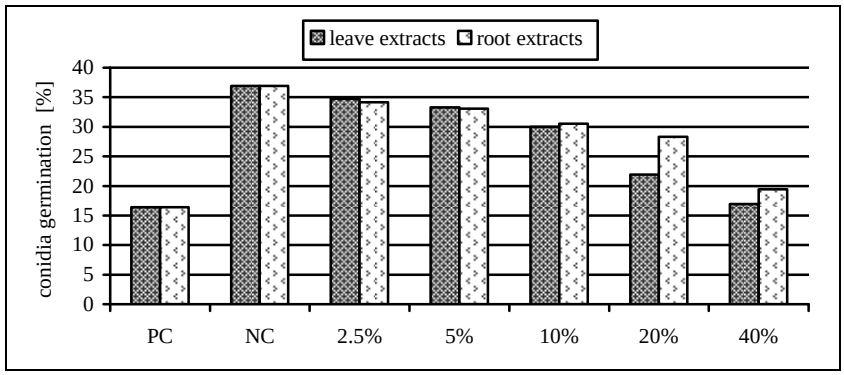

Fig. 1. Influence of the nettle extracts on the conidia germination of *Fusarium proliferatum*. PC - positive control, NC - negative control

The value of the conidia germination was at a level of from 34.15 to 19.43% when the extracts of nettle leaves were used, and from 34.73 to 16.95% after application of the extracts of nettle root. However, Topsin M 500SC used as the positive control inhibit the spore germination at the level 16.4%, while 36.9% in case of the negative control. For the both extracts, it has been observed that with the increase of theirs concentrations, the higher inhibition of the conidia germination was obtained (Fig. 1).

The percentage of noted reduction of the conidia germination amounted between 47.4% when applying 40% extract of nettle root extract to 7.5% (for 2.5% root extract) and 54.1% for 40% of the nettle leaves extracts to 5.9% (for 2.5% leaves extract) towards to the negative control, while stimulated compared to the positive control. However, the strongest inhibition of the spore germination of *F. proliferatum* was observed when Topsin M 500 SC was used.

Moreover the antifungal activity of the leaves and nettle root extracts towards *F. proliferatum* cultured on 2 different media was evaluated by the values of the growth rate index of the mycelium and the degree of the linear growth inhibition of fungi.

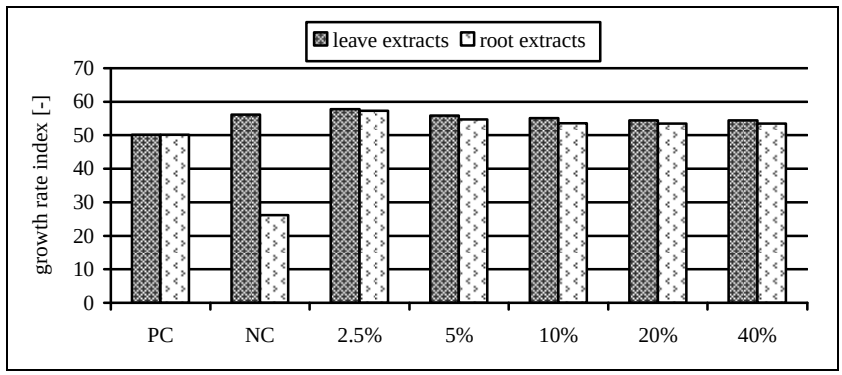

Fig. 2. Influence of the nettle extracts on the growth rate index of *F. proliferatum* on PDA medium. The symbols are in Figure 1

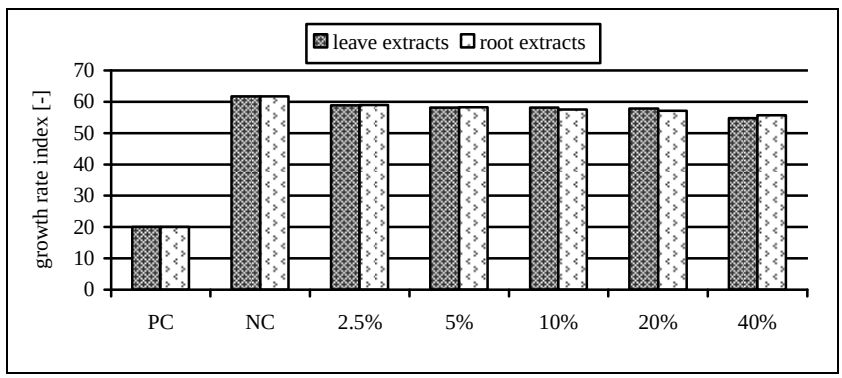

Fig. 3. Influence of the nettle extracts on the growth rate index of *F. proliferatum* on Tomato medium. The symbols as in Figure 1

The value of the growth rate index of *F. proliferatum* on PDA medium amounted from 53.46 to 57.31 for extracts of nettle root and from 54.39 to 57.79 for extracts of nettle leaves. The antagonistic activity of the both extracts were most effective on PDA medium when the nettle root extract at 40% concentration was applied. The percentage decrease was about 4.8% compared with the negative control. In contrast, the results obtained with nettle leave extract at the same concentration was lower and the percentage reduction was about 3.2% (Fig. 2). Similar activity of nettle extracts against *F. proliferatum* were obtained on Tomato medium, while the inhibitory efficacy of *U. dioica* was slightly higher. The value of the growth rate index amounted between 59.0-55.6 (for extracts of nettle root) and 58.8-54.8 (for extracts of nettle leaves). The decrease in the growth rate index was from 4.4 to 9.8% and from 4.6 to 11.2% compared with positive control, respectively. The

highest measured value of the growth inhibition was noted at 40% concentration both the extract of nettle root and extract of nettle leaves (Fig. 3).

Selective activity of *U.dioica* against the phytopathogenic fungi has been described in many research papers [5, 8, 13, 14, 17]. It has been proved that antifungal activity of nettle extracts is closely related to composition and concentrations of extracts, the sensitivity of spores and mycelium of the fungus. Hadizadeh et al [5] sugested that extract of nettle at the highest concentration of 0.9% markedly inhibited the mycelium growth of *A. radicina* completely (100%) or almost completely (97%) of *R. solani* and showed a relatively high degree (80%) of *F. oxysporum* and *F. solani*. Similarly Tapwal et al [13] found that the *U.dioica* can restrict the growth of *A. radicina* (41.6%), *R. solani* (18.8%) and *F. oxysporum* (20.1%) but at a higher concentration the leave extract *ie* 20%. Other studies [17] report that the ethanolic extract and their compounds had inhibitory activities on the growth rate (from 3.17 to 8.97%) and mycelial weight of *P. digitatum*.

On the other hand nettle herb extracts stimulated linear growth of colonies, biomass increment and formation of spores in *A. sclerotiorum* and *A. alternata*. In the case of *F. oxysporum* increasing the number of macroconidia was recorded only in the highest concentration of nettle extract, although the inhibition of the linear mycelial growth was observed [14]. Similarly Modarresi-Chahardehi et al [11] observed a different effect of nettle extract on the fungi, notably inhibition the germination of conidia *A. flavus*, *A. fumigatus*, *Penicillium* sp. and *Rhizopus* sp., while found no effects on mycelium. The resistance of fungal species against crude extracts of *U. dioica* could be due to their morphological structure, fungi have thicker cell walls and contain higher percentage of chitin. Furthermore inhibition of mycelial growth and production of morphological elements may significantly reduce the survival of pathogens in the soil [11, 18].

Conclusions

On the basis of conducted research one can draw the following observations:

1. Increased concentration the extracts of nettle leaves and nettle root inhibit linear growth of mycelium and spore germination of *F. proliferatum* compared to negative control, but increased compared to Topsin M 500 SC (positive control).
2. Nettle root extracts has demonstrated 2 fold more potent on the mycelium growth than the extract from the nettle leaves.
3. Much more intense effect (3-4 fold higher) compared to the linear growth revealed extracts on spore germination.
4. On the tomato medium inhibiting the linear growth of *Fusarium proliferatum* was 2-3 fold higher than on the PDA medium.
5. Due to the stronger effects of the Topsin M 500 SC should be carried out studies with higher concentrations of the nettle leaves and nettle root extracts of *U.dioica.*

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OCENA WŁAŚCIWOŚCI PRZECIWGRZYBOWYCH POKRZYWY ZWYCZAJNEJ WOBEC *Fusarium proliferatum*

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Abstract: Celem przeprowadzonych badań była ocena właściwości przeciwgrzybowych ekstraktów z liści i korzenia pokrzywy zwyczajnej wobec *Fusarium proliferatum*. Ocenę właściwości antagonistycznych ekstraktów w stężeniach 2,5, 5,0, 10, 20 i 40% przeprowadzono metodą hodowlano-płytkową z zastosowaniem podłoży PDA pomidorowego. Hodowlę prowadzono w temp. 26ºC przez 9 dni, dokonując pomiarów co 1-2 dni. Kontrolę pozytywną stanowił Topsin M 500 S.C, a kontrolę negatywną woda destylowana. Określono indeks kiełkowania zarodników, indeks tempa wzrostu oraz stopień redukcji liniowego wzrostu *Fusarium* sp. Uzyskane wyniki badań wykazały zahamowanie kiełkowania zarodników przez wszystkie zastosowane stężenia ekstraktów z korzenia pokrzywy (od 7,5 do 47,3%) oraz stężenia ekstraktów z liści pokrzywy (od 5,9 do 54,0%) w porównaniu do próby negatywnej, lecz stymulujące działanie w porównaniu do próby pozytywnej. Najwyższy stopień zahamowania liniowego tempa wzrostu obserwowano zarówno po zastosowaniu ekstraktu z korzenia, jak i liści na podłożu pomidorowym odpowiednio o 9,8 i 11,2%, a na podłożu PDA odpowiednio o 5,5 i 3,2% przy stężeniu 40% w porównaniu do próby negatywnej, lecz stymulujące działanie w porównaniu do próby pozytywnej.

Słowa kluczowe: pokrzywa zwyczajna, aktywność przeciwgrzybowa, *Fusarium proliferatum*