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Effect of foliar selenium application on the infestation of the perennial ryegrass *Lolium perenne* L. and tall fescue *Festuca arundinacea* Schreb. by fungal pathogen *Fusarium culmorum* (W.G. Sm.) Sacc.

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1 SUMMARY

Selenium is considered an essential element for the life of animals, including humans. Anyway, its necessity and function in the plant organism have not been fully explained yet. The aim of the work was to investigate an effect of foliar application of selenium on the infestation of the perennial ryegrass and tall fescue by fungal pathogen *Fusarium culmorum* based on a determination of ergosterol in plant biomass. Two major species of grass (perennial ryegrass and tall fescue) cultivated under defined climate chamber conditions were included in the experiment. Within 5 weeks from their germination, a solution of selenium in the form of selenite or selenate, corresponding to 4 mg/m^2 Se, was foliarily applied onto the plants. After 14 days of spraying, a solution containing conidia of *Fusarium culmorum* was applied to the plants. Subsequently, samples of green matter were taken at 14-day intervals, and the content of ergosterol and selenium were determined there. The content of ergosterol, which was selected as a marker of fungal pathogens, was found to be significantly higher ($P<0.05$) on the 28th day after the selenite and selenate application in both grass species. This increase was conclusive ($P<0.05$), when compared to the control group. No difference was observed between the selenium forms used. From our experiment, it is clear the plants of the perennial ryegrass and tall fescue were more easily attacked by fungal pathogen *Fusarium culmorum* after the application of selenium. Thus, it is possible to assume the application of selenium acts as stress a factor to plants.

2 INTRODUCTION

Perennial ryegrass *Lolium perenne* L. is the world's most widely used grass species for forage purposes (Wang *et al.,* 2016) and lawns (Knot *et al.,* 2017). It has been considered as a species requiring heat, sufficient moisture and nutrient supply in soil. It is characterized by high nutrients content, especially water-soluble carbohydrates (Skladanka *et al.,* 2014). Plants of perennial ryegrass are commonly infected with endophytes that may increase resistance to stress factors, i.e. infestation with fungi of genus *Fusarium* spp. (Wiewiora 2015). Another species widely used in forage and turf planting is a tall fescue *Festuca arundinacea* Schreb. It is

characterized by wide ecological amplitude and great adaptability to weather fluctuations (Skladanka *et al.,* 2014). Because of these positive features, it is often selected as a parent material for intergeneric hybrids (Humphreys *et al.,* 2014). Selenium (Se) is an essential element significantly influencing health status of animals and humans. The insufficient supply of organism with this element leads to many disorders. Conversely, higher intake can be toxic (Rahman *et al.,* 2015; Owosu-Sekyere *et al.,* 2013). Selenium, as a part of selenoproteins, regulates the antioxidant system and thus prevents the oxidative destruction of biological membranes and prevents the damage of the body by heavy metals. As a result of the involvement of selenium compounds in numerous biological functions, its deficit impairs overall health status of animals, increases the susceptibility of juveniles to infectious diseases, causes reproductive disorders or may be direct cause of an illness (Horky, 2014; Wang *et al.,* 2016). Selenium concentration of plant biomass is derived from its soil content and may vary considerably depending on the region (Guerrero *et al.,* 2014). Its effect on plants is not fully understood at present but according to a number of authors (Kaur and Nayyar, 2014; Djanaguiraman *et al.,* 2005; 2010) selenium increases the resistance of plants to abiotic and biotic stresses and acts as a growth regulator and antioxidant. A number of studies have found that adequate selenium supplementation has increased the growth rate of plants (Hartikainen *et al.,* 2000; Xue 2001). On the other hand, excessive selenium doses may act as a stress factor (Hartikainen *et al.,* 2000). The sensitivity of plants to high selenium doses varies depending on the species and environmental conditions. Significant decrease in dry matter yield was observed above a level of 5μg $Se.g^{-1}$ soil in raya *Brassica juncea* Czern L., and maize *Zea mays* L., 4 μg $Se.g^{-1}$ soil in wheat *Triticum aestivum* L. and 10μg $Se.g^{-1}$ soil in rice *Oryza sativa* L. shoots (Rani *et al.,* 2005). Plants may be attacked by a number of fungal pathogens. A frequent cause of grass and cereal diseases leading to the production of mycotoxins is *Fusarium* ssp. These pathogens significantly reduce yields and the quality of the infected crops, and the production of mycotoxins directly threatens the health of livestock and humans and causes considerable economic losses. Ergosterol is a fungal indicator which offers an efficient measure of living fungal biomass (Kim and Vujanovic, 2016; Wambacq *et al.,* 2016). The aim of the work was to investigate the effect of foliar application of selenium on the infestation of the perennial ryegrass and tall fescue by fungal pathogen *Fusarium culmorum* (W.G. Sm.) Sacc. based on determination of ergosterol in plant biomass.

3 MATERIALS AND METHODS

3.1 Plants and Infection: The species tall fescue (*Festuca arundinacea*, Zuzana variety) and perennial ryegrass (*Lolium perenne*, Kertak variety) were used in the experiment. The experiment was carried out in a climate chamber under defined conditions - daytime temperature 24 °C (from 6 a.m. to 6 p.m.), night temperature 20 °C (6 p.m. to 6 a.m.), light day length 12 hours, light intensity 380 $\mu mol \cdot m^{-1} \cdot s^{-1}$, relative humidity 65%. Each pot (10 cm × 10 cm × 10 cm) was filled with substrate (600 g) and sown with seeds (0.05 g seed/pot). In each group were realized three repetitions. The green mass from each repetition was analysed separately. The pots were stored in a climate chamber and plants regularly watered. Four weeks after sowing, the plants were cut to a height of about 3 cm to thicken the stand. Foliar application of selenium was performed after 7 days. Two forms - sodium selenite and sodium selenate were used. A solution of a dose of 4 mg/m^2 of Se was prepared. *Fusarium culmorum* BCCO strain 20_0210, grown on potato dextrose agar from active culture, was used to induce infection. After 14 days of selenium application, the leaf tops were trimmed to disturb their integrity and to applied to the suspension of *Fusarium* conidia.

Subsequently, samples of green matter were taken at 14-day intervals, weighed and dried. The intensity of plant infestation by pathogen *Fusarium culmorum* was monitored by determining the content of ergosterol.

3.2 Sample preparation: The dry plant samples were grinded. 0.5 g of grinded samples was mixed with 75% solution of HPLC grade methanol (Sigma-Aldrich, St. Louis, MO, USA) in Falcon 15 mL Conical Centrifuge Tubes (Corning, Tewksbury, MA, USA). Extraction of samples was performed by Multi Reax shaker (Heidolph, Schwabach, Germany) for 24 hours. Samples were centrifuged by a MPW 223a centrifuge (MPW, Warsaw, Poland) for 10 minutes in frequency of 1024 rounds per minute. Supernatant from centrifuged samples was and filtrated by Nalgene 25mm Syringe Filters 0.45 µm (Thermo Fisher Scientific, Waltham, MA, USA). 1 ml of each filtrated sample was transferred to 50ml volumetric flask and diluted by 10 ml of distilled water. The volume of 1 ml of 2N Folin & Ciocalteu's phenol reagent (Sigma-Aldrich, St. Louis, MO, USA) was added to samples, after 5 minutes fallowed by 10 ml of 7% Sodium carbonate (Sigma-Aldrich, St. Louis, MO, USA). Volumetric flasks were filled up by distilled water, mixed and left for 90 minutes.

3.3 Determination of ergosterol: Ergosterol was extracted from leaf litter by 30 min refluxing in alcoholic base and purified by solid-phase extraction. Calibration solutions were prepared by dissolving of Gallic acid for titration (Sigma-Aldrich, St. Louis, MO, USA) by 75% solution of HPLC grade methanol (Sigma-Aldrich, St. Louis, MO, USA) in 50ml volumetric flasks. Concentration of calibration solutions were: 1 mg/ml; 0.5 mg/ml; 0.25 mg/ml; 0.1 mg/ml; 0.05 mg/ml and 0.025 mg/ml. 1 ml of each calibration sample was transferred to 50ml volumetric flask and diluted by 10 ml of distilled water. The volume of 1 ml of 2N Folin & Ciocalteu's phenol reagent (Sigma-Aldrich, St. Louis, MO, USA) was added to samples, after 5 minutes followed by 10 ml of 7% Sodium carbonate (Sigma-Aldrich, St. Louis, MO, USA). Volumetric flasks were filled up by distilled water, mixed and left for 90 minutes. All used distilled water was prepared by Ultrapore Simplicity Water Purification System type 1 (Merck Millipore, Billerica, MA, USA). Samples were filled into quartz cell SM/Q/10 (Exacta + Optech, San Prospero, Italy). Measurement of visible absorbance in wavelength of 765 nm was made by UV/VIS Spectrophotometer Lambda 25 (Perkin Elmer, Waltham, MA, USA). The concentration of samples was calculated from calibration line. The resulting concentration of original solid plant samples were calculated using formula:

$$m = \frac{c_g \times V_c \times m_r \times V_{ex}}{m_s \times V_p}$$

m is weight of phenolic compounds in 100 g of original dry plant sample (g), c_g is concentration of phenolic acid in measured sample received from calculation from calibration line (g/ml), V_c is volume of calibration solutions used for reaction (1 ml), m_r is weight of reference sample used for calculation (100 g), V_{ex} is volume of extraction solution (10 ml), m_s is weight of sample used for extraction (g) and V_p is volume of extracted sample used for reaction (1 ml).

3.4 Statistical analysis: The data were processed statistically using STATISTICA.CZ, version 10.0 (the Czech Republic). The results were expressed as mean ± standard deviation (SD). Statistical significance was determined by examining the basic differences between control groups and variants treated with selenate or selenite of both experimental species using ANOVA and Scheffé's test (one-way analysis). The differences with $P<0.05$ were considered to be significant.

4 RESULTS AND DISCUSSION

Ergosterol is a part of cell walls of moulds and fungi, and therefore the determination of its content is used as an indirect parameter of total fungal biomass in the samples. However, the ergosterol content of the samples does not have to correspond to the amount of detected mycotoxins and cannot be used even as a marker for their tentative determination[17]. The observed ergosterol, which was chosen as a marker of fungal pathogen infestation, was found to have significantly higher ($P < 0.05$) content on the 14th day after *Fusarium culmorum* infection in a perennial ryegrass. This increase was 66.7% compared to the control group. No significant difference was found in the tall fescue, compared to the control group or between the two forms of selenium (Fig. 1A). Samples obtained on the 28th day after *Fusarium culmorum* infection already showed a significant ($P < 0.05$) increase in ergosterol content in selenium treated variants in both species. In the case of perennial ryegrass, the ergosterol content after selenate treatment was higher by 80.7%, after selenium treatment even by 147.0% higher compared to the control group. In the biomass of tall fescue, a rise in ergosterol content after selenate application by 202.6% and by 271.0% after selenite application was observed. There was no significant difference between used forms of selenium (Fig. 1B).

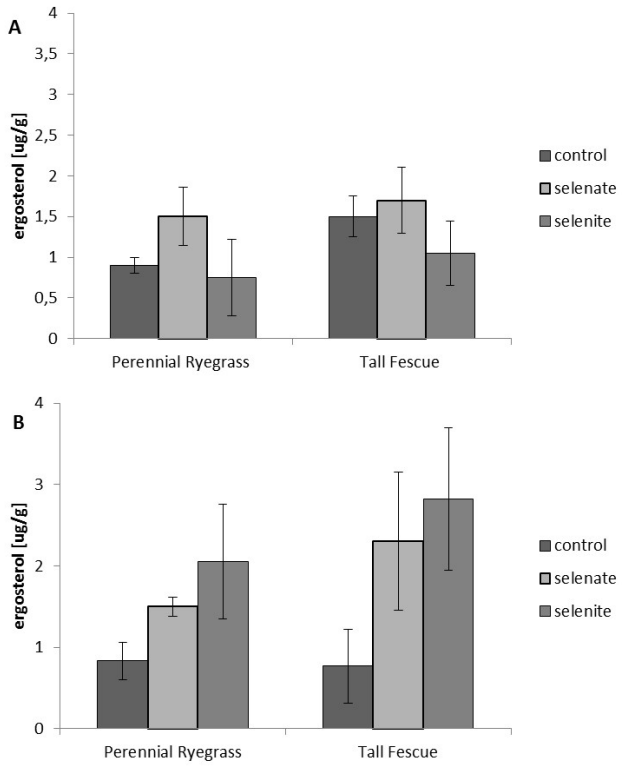

Fig. 1: Content of ergosterol 14th day (A) and 28th day (B) after application of conidia of *Fusarium culmorum*

Selenium is part of a number of enzymes and proteins with antioxidant activity[7,18] and its presence in the plant can bring a number of benefits. Some authors (Betancor *et al.,* 2015; Kumar *et al.,* 2014; Klusonova *et al.,* 2015) state selenium supplementation can increase resistance of plants to abiotic and biotic stresses and act as a growth regulator and antioxidant. On the other hand, selenium can be toxic to plants. It causes chlorosis, reduces

carbohydrate, protein and antioxidant content and deteriorates oxidative stress and methylglyoxal toxicity. These negative effects are particularly noticeable when high selenium doses are used (Ding *et al.,* 2017). Our results show that plants treated with any form of selenium were more affected by fungal diseases (Mostofa *et al.,* 2017). In mentioned publication was confirmed that selenium acted as a stress factor reducing the resistance of plants to fungal pathogens. On the other hand, the application of selenium did not eliminate plant growth, which was not only negatively affected but was even higher after selenium was applied ($P < 0.05$), (Fig. 2A, 2B).

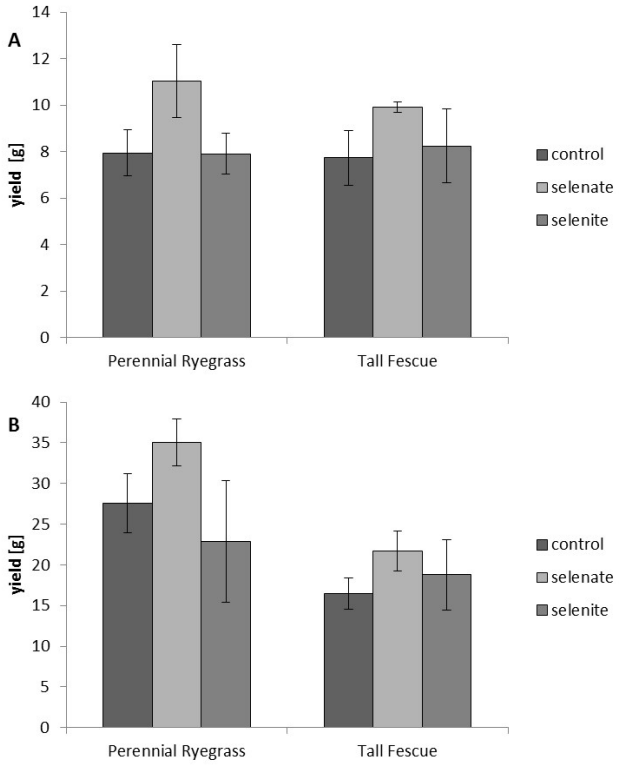

Fig. 2: Yield of dry biomass 14th day (A) and 28th day (B) after application of conidia of *Fusarium culmorum*

Dohnal *et al.* (2007) determined the grass species with fine leaves, such as perennial ryegrass, are more susceptible to fungal diseases, while tall fescue belongs to species that are more resistant. Increased plant resistance to fungal pathogens can also be achieved by symbiosis with endophytic fungi, which is very common in grasses (Panka *et al.,* 2013; Mika *et al.,* 2005). The results coming from our observation are not statistically significant but may indicate a lower resilience of the tall fescue due to selenium application and consequently, its higher susceptibility to fungal pathogen infestation.

4 CONCLUSION

From our experiment, it is clear that the plants of the perennial ryegrass and tall fescue were more easily subjected to fungal pathogen *Fusarium culmorum* after the application of selenium. However, the infestation was progressively increasing from the application of selenium and the inducement of infection with conidia of *Fusarium*, content of ergosterol was increase up to 217 % in compare with control group. Therefore, it is possible to assume that

selenium application affects plants as a stress factor increasing the susceptibility of plants to fungal pathogens.

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6 REFERENCES

Betancor MB, Almaida-Pagán PF, Sprague M, Hernández A and Tocher DR: 2015. Roles of selenoprotein antioxidant protection in zebrafish, Danio rerio, subjected to dietary oxidative stress. *Fish Physiology and Biochemistry*, 41: 705.

Ding Y, Wang Y, Zheng X, Cheng W, Shi R and Feng R: 2017. Effects of foliar dressing of selenite and silicate alone or combined with different soil ameliorants on the accumulation of As and Cd and antioxidant system in *Brassica campestris*. *Ecotoxicology and Environmental Safety,* 142: 207.

Djanaguiraman M, Devi DD, Shanker AK, Sheeba JA and Bangarusamy U: 2005. Selenium – an antioxidative protectant in soybean during senescence. *Plant and Soil*, 272: 77.

Djanaguiraman M, Prasad PVV and Seppanen M: 2010. Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defence system. *Plant Physiology and Biochemistry*, 48: 999.

Dohnal V, Kaderova I, Jezkova A and Skladanka J. 2007: Obsah ergosterolu u vybraných druhů trav na konci vegetačního období. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 4: 9.

Guerrero B, Llugany M, Palacios O and Valiente M. 2014: Dual effects of different selenium species on wheat. *Plant Physiology and Biochemistry,* 83: 300.

Hartikainen H, Xue T and Piironen V. 2000: Selenium as an anti-oxidant and pro-oxidant in ryegrass. *Plant Soil*, 225: 193.

Horky P. 2014: Influence of increased dietary selenium on glutathione peroxidase activity and glutathione concentration in erythrocytes of lactating sows. *Annals of Animal Science,* 14: 869.

Humphreys MW, O'Donovan SA, Farrell MS, Gay AP and Kingston-Smith AH. 2014: The potential of novel Festulolium (2n = 4x = 28) hybrids as productive, nutrient-use-efficient fodder for ruminants. *Food and Energy Security,* 3: 98.

Kaur N, Sharma S and Nayyar H. 2014: Selenium in Agriculture: A Nutrient or Toxin for Crops? Archives of *Agronomy and Soil Science*, 60: 1593.

Kim SH and Vujanovic V. 2016: Relationship between mycoparasites lifestyles and biocontrol behaviors against *Fusarium* spp. and mycotoxins production. *Applied Microbiology and Biotechnology*, 100: 5257.

Klusonova I, Horky P, Skladanka J, Kominkova M, Hynek D, Zitka O, Skarpa P, Kizek R and Adam V. 2015: An Effect of various selenium forms and doses on antioxidant pathways at clover (*Trifolium pratense* L.). *International Journal of Electrochemical Science,* 10: 9975.

Knot P, Hrabe F., Hejduk S, Skladanka J, Kvasnovsky M, Hodulikova L, Caslavova I and Horky P. 2017: The impacts of different management practices on botanical composition, quality, colour and growth of urban lawns. *Urban Forestry & Urban Greening*, 26: 178.

Kumar A, Singh RP, Singh PK, Awasthi S, Chakrabarty D, Trivedi PK and Tripathi

RD. 2014: Selenium ameliorates arsenic induced oxidative stress through modulation of antioxidant enzymes and thiols in rice (*Oryza sativa* L.). *Ecotoxicology*, 23: 1153.

Mika V, Kuban V, Klejdus B, Odstrcilova V and Nerusil P. 2005: Phenolic compounds as chemical markers of low taxonomic levels in the family *Poaceae*. *Plant Soil and Environment*, 51: 506.

Mostofa MG, Hossain MA, Siddiqui MN, Fujita M and Tran LP. 2017: Phenotypical, physiological and biochemical analyses provide insight into selenium-induced phytotoxicity in rice plants. *Chemospherem*, 178: 212.

Owusu-Sekyere A, Kontturi J, Hajiboland R, Rahmat S, Aliasgharzad N, Hartikainen H and Seppänen MM. 2013: Influence of selenium (Se) on carbohydrate metabolism, nodulation and growth in alfalfa (*Medicago sativa* L.). *Plant and Soil*, 373: 541.

Panka D, Piesik D, Jeske M and Baturo-Ciesniewska A. 2013: Production of phenolics and the emission of volatile organic compounds by perennial ryegrass (*Lolium perenne* L.) /*Neotyphodium lolii* association as a response to infection by *Fusarium poae*. *Journal of Plant Physiology,* 170: 1010.

Rahman MM, Erskine W, Materne MA, McMurray LM, Thavarajah P, Thavarajah D and Siddique KHM. 2015: Enhancing selenium concentration in lentil (*Lens culinaris* subsp. *culinaris*) through foliar application. *The Journal of Agricultural Science,* 153: 656.

Rani N, Dhillon KS and Dhillon SK. 2005: Critical levels of selenium in different crops grown in an alkaline silty loam soil treated with selenite-Se, *Plant and soil*, 277: 367.

Skladanka J, Cagas B, Dolezal P, Havlicek Z, Hejduk S, Horky P, Jancovic J, Klusonova I, Knot P, Kovar P, Mejia JEA, Mikyska F, Nawrath A, Pokorny R, Slama P, Szwedziak K, Tukiendorf M, Seda J, Vozar L, Vyskocil I, Zeman L. 2014: *Picninarstvi*. (Mendelova univerzita v Brne, Brno) 112.

Stanisz E, Zgoła-Grzeskowiak A, Waskiewicz A, Stępien Ł and Beszterda M. 2015: Can ergosterol be an indicator of *Fusarium* fungi and mycotoxins in cereal products? *Journal of the Brazilian Chemical Society*, 26: 705.

Wambacq E, Vanhoutte I, Audenaert K, De Gelder L and Haesaert G. 2016: Occurrence, prevention and remediation of toxigenic fungi and mycotoxins in silage: a review. *Journal of the Science of Food and Agriculture*, 96: 2284.

Wang J, Cogan NOI, Forster JW and Rognli OA. 2016: Prospects for applications of genomic tools in registration testing and seed certification of ryegrass varieties. *Plant Breeding,* 135: 405.

Wiewiora B, Zurek G and Zurek M. 2015: Endophyte-mediated disease resistance in wild populations of perennial ryegrass (*Lolium perenne*). *Fungal Ecology*, 15: 1.

Xue T, Hartikainen H and Piironen V. 2001: Antioxidative and growth-promoting effect of selenium on senescing lettuce. *Plant Soil*, 237: 55.