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LETTER TO THE EDITOR

Update the comments on "Study of biological activity of *Tricholoma equestre* fruiting bodies and their safety for human"

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Dear Editor,

In the letter to the Editor, Rzymski et al. [1] commented on Muszyńska et al.'s paper about *Tricholoma equestre* (L.) P. Kumm properties [2] but an ongoing debate of *T. equestre* safety is very long.

It is incomprehensible intentions of Rzymski et al. [1] which are presented in letter to your journal about paper by Muszyńska et al. [2]. All aspects in his letter were respected in our research and presented in our publication.

Muszyńska and team in 2005 started the first research with *T. equestre* fruiting bodies and mycelium and the first publication about *T. equestre* and their cultures in vitro was published in 2009 [3].

Our paper [2] published in European Food Research and Technology was based on Moukha et al. [4] (references list in Muszyńska et al. [2]). *T. equestre* is a species with a wide range of occurrence, its fruiting bodies can be found in Europe, North America, Asia (Japan), and Central America and Africa. According to genetic research, *T. equestre* species complex includes three ectomycorrhizal species *Tricholoma flavovirens* (Pers.) S. Lundell, *Tricholoma auratum* (Paulet) Gillet, and *T. equestre* (L.) P. Kummer. *T. equestre* as a typical ectomycorrhizal is associated with *Pinus sylvestris* or *Abies alba* [4]. In Poland, *T. equestre* occurs in coniferous and mixed forest and this mushroom is in commercial sale in Poland.

In many European countries, this species is considered as dangerous because of the cases of toxic activity, which were reported from 2001 to 2017, with a number of fatalities. Acute poisoning caused by *T. equestre*, including lethality, was reported from France, Spain, Poland, Lithuania and other countries [5–9].

Nieminen et al. [9] reported in mice exposed to 12 g/kg for 28 days (which corresponds to the dose of 960 g for 80 kg man) of *T. equestre* freshly frozen mushroom, higher plasma bilirubin content and higher creatine kinase activity than in the control mice. Rise in the creatine kinase level was one of the effects of research in which *T. equestre* was given to the mice. It is documented that such raise is the symptom which precedes massive rhabdomyolysis. Moreover, the authors showed an increased incidence of pericardial inflammation in mice after the *T. equestre* diet [9]. In our study, we confirmed this observation after in vitro experiments but we did not present the mechanism of rhabdomyolysis as suggested by Rzymski et al. [1].

In Poland, deadly poisoning with *T. equestre* has also been documented [7, 8, 10, 11]. The cause of the poisoning was probably rhabdomyolysis, which results in damage of the cell membranes in the skeletal muscles. Intoxication of *T. equestre* may be connected with the high mortality rate of about 20% [11].

Chodorowski et al. [7, 8] reported that *T. equestre* causes poisoning among children and adults although there is no evidence that shows which compound is responsible for this phenomenon. On the other hand, *T. equestre* is moderately rich in substances acclaimed as necessary for an organism. The clinical symptoms of the poisoning depend on persons' age, time of consumption and amount of the mushrooms that were eaten. Acute respiratory failure and myocarditis with cardiac arrhythmia and

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cardiovascular collapse happened with different frequency [7, 8].

In Lithuania, clinical findings showed evidence of rhabdomyolysis after consumption of *T. equestre*. Based on the laboratory data tests, an elevation of creatine kinase, aspartate aminotransferase and alanine aminotransferase was observed in patients' serum [12]. Hayakawa et al. [13] reported data from Japan of the poisoning of the *Tricholoma* species.

Muszyńska et al. [3] investigated the content of indole compounds in this species. It has been shown that the content of these metabolites is very different in quantitative terms in *T. equestre* and varies widely between 0.01 and 34.11 mg/100 g d.w. (dry mass).

Tricholoma equestre is also a species of mushroom, with the highest content of sodium. The content of sodium (26.80 mg/100 g d.w.) in this species is two times higher than in other species of Basidiomycota. The amount of zinc determined in *T. equestre* fruiting bodies after the digestion time ranged from 1.11 to 6.83 mg/100 g d.w. but in *T. equestre* from in vitro cultures was higher and ranged from 1.52 to 14.4 mg/100 g d.w. The above studies may suggest that this species can be a good source of zinc in the diet (because the daily requirement for the human body is 12 mg) and can even cover the daily requirement for this element or be higher and toxic (according to the FAO/WHO standards). Zinc poisoning is an impaired oxygen transport that can lead to damage to the striated muscles [14].

In the research of Muszyńska et al. [2], it was recognized that *T. equestre* extracts may promote pro-inflammatory signaling comparing to many experiments in which the anti-inflammatory properties of edible mushrooms were presented [14–17]. In our study, after *T. equestre* treatment in A549 cells we observed statistically significant increase of COX-2 and a statistically significant decrease of Nrf2 protein level, which suggested pro-inflammatory properties of *T. equestre* extracts. The information why we used ethanol in molecular research is because of alcoholatures are one of main preparation as medicines from natural products.

Rzymski et al. [1] showed also the big problem: if mycologists are not good in identifying mushroom species, what with case of people who are collecting *Tricholoma* species in forest without genetic tools. We have a lot of edible mushrooms which consumption is safe without any health consequence for humans; therefore, controversial *T. equestre* species, with unclear culinary and medicinal quality, should not be promoted.

In conclusion, the phylogenetic relationship observed between these species from *T. equestre* complex suggests that further toxicological studies are necessary. Moreover, what is very important, White et al. [18] proposed a new clinical classification of mushroom poisoning which includes *Tricholoma* spp. as 3B caused syndromes with rhabdomyolysis as the primary feature with delayed onset.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This paper does not contain any studies with human or animal subjects.

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