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CONSIDERATIONS REGARDING THE *TRITICUM* PHYTOTOXICITY TEST

Oana Alexandra Drăghiceanu [1], Irina Fierăscu [2], Radu Claudiu Fierăscu [2], Roxana Bradzis [2], Codruța Mihaela Dobrescu [1,*], Liliana Cristina Soare [1]

[1] University of Pitesti, Department of Natural Sciences, 1 Targu din Vale street, 110040, Pitesti, Arges, Romania
[2] National Institute for Research & Development in Chemistry and Petrochemistry – ICECHIM Bucharest, Emerging Nanotechnologies Group, 202 Spl. Independentei, 060021, Bucharest, Romania

Abstract
The test conducted on wheat (Triticum aestivum) is frequently used as a prescreening test for phytotoxicity studies in higher plants due to its advantages: quick results, simplified operative procedure, good reproducibility and repeatability and reduced costs.
The laboratory methodology for the Triticum test (Constantinescu method) is applied by different authors with some modifications imposed by the properties of the tested substance (structural formula, purity, solubility in water or other solvents, stability in water or solvent, and biodegradability).
The potential of the genus Triticum to be used for toxicity tests is demonstrated by the numerous research studies that evaluated different categories of substances determining different morphological, physiological, biochemical and genetic parameters.

Keywords: toxicology, toxicity test, Triticum sp.

1. INTRODUCTION

The history of toxicity studies begins with Paracelsus (1493-1541), who demonstrated the toxic and beneficial effects of toxins and the connection between dose and response in the case of medication (Parasuraman, 2011). The response to the dose range is the basis of toxicology and medicine and a fundamental aspect in agriculture, biology, ecology and physiology (Agathokleous et al., 2018). The term *toxicology* was introduced in the 17th century (Milles, 1999).

Toxicology studies the toxic substances (origin, physical, chemical and biological properties, isolation, identification and dosage) together with their mechanism of action and the measures taken to prevent their harmful effects (Drochioiu et al., 2013). Thus, different testing methods (toxicology tests) were devised for and applied to both aquatic and terrestrial species to determine the effects of toxic substances that might be potentially dangerous to the living organisms.

These methods are periodically updated (Table 1): the OECD (Organization for Economic Cooperation and Development) 201 Freshwater Alga and Cyanobateria, Growth Inhibition Test was elaborated in 1981 and since then it has been modified/ improved/ upgraded several times. The most recent modification was made on 28.07.2011. The variant of the OECD 2008 test - Terrestrial Plants, Growth Test - developed in 1984 was updated on 19.07.2006.

*Corresponding author, E-mail address: codrutza_dobrescu@yahoo.com

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Table 1. Examples of the most common test applied for aquatic and terrestrial phytotoxicity

Kind of Test	Species / Parameters	Protocols / guidelines	Latest version
Aquatic	*Lemna*	USEPA OCSPP 850.4400 - Aquatic Plant Toxicity Test Using *Lemna* spp.	June 2012
		OECD 221 :*Lemna* sp. Growht Inhibition Test	March 2006
	Alga / Cyanobacteria	USEPA OCSPP 850.4500 - Algal Toxicity USEPA OCSPP 850.4550 - Cyanobacteria (*Anabaena* flos-aquae) Toxicity	June 2012
		OECD 201: Freshwater Alga and Cyanobateria, Growth Inhibition Test	July 2011
Terrestrial	Vegetative Vigor	OECD 227- Terrestrial Plant Test: VegetativeVigour Test	July 2006
		USEPA OCSPP 850.4150 - Vegetative Vigor	June 2012
	Seedling Emergence and Seedling Growth	OECD 208 - Terrestrial Plants, Growth Test	July 2006
		USEPA OCSPP 850.4100 - Seedling Emergence and Seedling Growth	June 2012
	Early Seedling Growth	USEPA OCSPP 850.4230 - Early Seedling Growth Toxicity Test	June 2012

(OECD, 2018; USEPA, 2012)

2. PHYTOTOXICITY

"Phytotoxicity" refers to the harmful deviations (according to the visual and measured evaluations) from the normal appearance and growth of plants as a response to a given chemical substance (OCDE, 2006).

Phytobiological tests are used in toxicity screening and bioremediation tests to offer information for the evaluation of cytotoxicity and genotoxicity.

The aim of phytobiological tests is to quantify the effects of certain natural or synthetic compounds on vegetative growth during the established testing period, based on the evaluation of the selected measurement variables (root and stem length, fresh and dry mass weight for root and stem, water content, etc.).

The following characteristics are taken into account in the selection of suitable test species:

- the species have even seeds, which are readily available and produce a regular reliable and uniform germination as well as a uniform growth of seedlings;
- the plant is adapted to laboratory tests and can generate reliable and reproducible results in different testing centers;
- the sensitivity of the tested species must be similar with the response of the plants found in the medium exposed to the chemical substance;
- their use, to a certain extent, in previous toxicity tests, for example in biotests for herbicides or heavy metals, salt or mineral stress tests or allelopathy tests and confirmation of their sensitivity to a wide range of stressing agents;
- compatibility with the growth conditions of the test method;
- compliance with the validity criteria of the test.

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In 1996, USEPA (United States Environmental Protection Agency) recommended the use of a series of plants of economic and ecological importance as biomarkers for toxicity evaluation in terrestrial and aquatic ecosystems (Parlak, 2016).

Usually, agricultural species are used for terrestrial ecosystems. However, they are not constantly more or less sensitive to the tested herbicides than the uncultivated species (Clark et al., 2004).

The parameters determined during the toxicity tests may be classified into:

- **quantitative measurements**: the number of emerged seedlings, the time necessary for their emergence, the survival percentage rate, seedling height, rootlet/ root length, dry mass of superterranean parts and root, etc.
- **semi-quantitative** or **qualitative observations** (observed or evaluated as percentage) include: visual observations of abnormal modifications that may appear in growth, development or morphology compared with a sample, etc. (Hooper and Anderson, 2009).

For example, the polyphenolic profile of *Triticum* seedlings was evaluated both qualitatively (using thin layer chromatography) and quantitatively (using spectrophotometric determination), the latter showing a decrease in the biosynthesis of polyphenolic derivatives in parallel with the increase in both the amount of bioactive substance and contact time (Rotariu, 2012).

Besides the conditions that must be met for the tests, these protocols provide the list of the recommended plant species. At present, the list of species suggested by OECD is composed of 32 cultivated species (9 monocotyledonous species and 25 dicotyledonous species) and 52 non-agricultural/wild/uncultivated species (García-Cortés et al., 2018).

3. ECOTOX

ECOTOX is an ecotoxicologic database created by USEPA, which offers information about the side effects of chemical stress factors on ecologically relevant aquatic and terrestrial species. It includes approximately 910,400 entries; the data are taken from specialist literature as well as from research collections published in different open access/ non-open access journals, which belong to the member states of the EPA, U.S. Geological Survey, OECD etc. The last update was performed in June 2018.

Out of the 673 publications focused on wheat, the oldest date back from 1929 and the latest from 2018, which indicates that the preoccupation about the influence of different factors on wheat are topical and of great importance. The predominant species analyzed in these studies is *Triticum aestivum* (approximately 80%). The parameter determined and evaluated in the first studies conducted on *Triticum aestivum* (Young, 1929; Murphy, 1929) was seed germination. Later, other aspects were taken into account:

- **morphological, growth, development and reproduction aspects:** root (Xie et al., 2010a) and stem length, root and stem weight (Sangeeta et al., 2018), number of deteriorated roots (Stanton and Fisher, 1985), cell division rate (Weinberger and Murthy, 1985), growth rate (Yang et al., 2003), vegetative reproduction (Deepak and Agrawal, 1999), number of leaves per plant (Dear et al., 2006), vigour (Calvelo Pereira et al., 2010), number of roots (Li et al., 2011), number of ears (Singh and Singh, 1989);
- **genetic aspects:** nuclear (Chen et al., 1973) and mitotic anomalies, chromosomal aberrations (Xie et al., 2010);
- **biochemical aspects:** water content (Lima et al., 2015), sugar content (Yadav and Yadav, 2003), enzymatic activity (Chen and Cai, 2015), secondary metabolism (Opriş et al., 2013), protein synthesis (Burrows, 1978), protein content (Menegat et al., 2103), N, P, and K content (Cakmak et al., 2009), non-enzymatic antioxidants – phenols (Hassan, 2007) and proline (Zhang et al., 2011);

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- **physiological aspects:** photosynthesis rate (Opriş et al., 2013), respiration rate (Pal Singh et al., 2008), water absorption (Inagaki et al., 2009), frost resistance (Freyman and Hamman, 1979), photosynthetic pigments (Chen and Cai, 2015), photosystem II activity (Opriş et al., 2013), and transpiration (Li et al., 2011).

The test on wheat (*Triticum aestivum*) is frequently used as a prescreening test for phytotoxicity studies in higher plants due to its advantages: quick results, simplified operative procedure, good reproducibility and repeatability and reduced costs.

The laboratory methodology for the *Triticum* test (Constantinescu method) was applied by different authors with some modifications imposed by the properties of the tested substance (structural formula, purity, solubility in water or other solvents, stability in water or solvent, and biodegradability). Due to the fact that the experiments may also be performed in the dark, they are extremely useful for testing photosensitive compounds (Wang, 1991).

The advantages of the varied methodology were increased by the posibility of preparing cultures in different media. Thus, Li et al. (2019) immersed the seeds in test solutions at the beginning of the experiment and after 7 days they made morphological (germination percentage, shoot length, etc.), biochemical (protein and chlorophyll content, etc.) and molecular (expression analysis of gene related to biosynthesis of indole-3-acetic acid) determinations.

Saleh et al. (2019) cultivated the seeds in pots with soil and after a week they applied NiO nanoparticles in suspension form. The harvesting of the tested biological material was done after 3 weeks from exposure.

Ma et. al (2019) used a hydroponic system to study Sb accumulation, translocation and phytotoxicity. They used Hoagland solution with different Sb concentrations; the solution was changed every three days to maintain a constant concentration.

In order to determine the effect of sewage sludge on the heavy metal concentrations in wheat, Shahbazi et al. (2017) mixed the amount of sludge specific to each sample with 6 kg of soil before planting.

Ştefănescu et al. (2017) developed the *Triticum* phytobiological method as an alternative method to determine *in vivo* acute toxicity introducing a new and original quantitative parameter: the inhibition concentration 50% (IC50), calculated through the graphical method of regression curves. This parameter was conceived as an alternative to the *in vivo* parameter, the lethal dose 50% (LD50). The aim of this study was to validate the method for determining *in vivo* acute toxicity using plant material instead of laboratory animals.

Using the *Triticum* test, researchers tested substances such as pesticides (Rangwala et al., 2013), PAHs (Paková et al., 2006), heavy metals (Cd, Cr, Cu, Pb, Mg, Ni, Se, Zn) (Rezapour et al., 2018), pharmaceutical, personal care products (Opriş et al., 2013), nanoparticles (Saleh et al., 2019), etc.

Gutu et al. (2015) used the modified Constantinescu method to establish the correlation between the growth of wheat seedlings and the absorption of two inorganic arsenic compounds; cariopses were exposed to substances only after their germination, when the root was approximately 1 cm in length.

The same method was applied to test extracts of *Cuscuta* (Ancuceanu et.al., 2005), polyphenolic extracts from coriander, variety *Omagiu* (Trifan, 2012), extracts of *Abutilon theophrasti* (Dinu et. al., 2012), hydroalcoholic root extracts of the species *Glycyrrhiza glabra* (fam. Fabaceae) and *Withania somnifera* (fam. Solanaceae) obtained using 70% ethanol and a hydrosoluble extract of commercial origin obtained from the root of the species *Panax notoginseng* (fam. Araliaceae) (Ancuceanu et. al., 2014), extracts of *Cirsium arvense* (Radu, 2014; Anghel et. al., 2014), extracts of *Panax notoginseng* and oat bran (Ancuceanu et. al., 2016), leaf extracts of *Amaranthus*

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retroflexus (Dinu et. al, 2017) or other synthetic chemical substances (Apostol et.al., 2014; Nicolescu et.al., 2014).

4. CONCLUSIONS

The evaluation of phytotoxicity for some substances such as pesticides, PAHs, heavy metals, nanomaterials and different extracts has been made using *Triticum aestivum* as a test plant ever since 1929. The first biological parameter monitored during this test was the germination percentage. Subsequently, other determinations of morphological, physiological, histological, biochemical and genetic parameters were performed. The current research introduced a new quantitative parameter - IC50, for root growth, as an alternative method to the *in vivo* parameter – lethal dose 50% (LD 50).

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*Corresponding author, E-mail address: codrutza_dobrescu@yahoo.com

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*Corresponding author, E-mail address: codrutza_dobrescu@yahoo.com