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Application of Phytotoxkit in the quick assessment of ashes suitability as fertilizers in sorghum crops**

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A b s t r a c t. The aim of the research was to check the suitability of the Phytotoxkit test for quick assessment of usefulness of ashes from the combustion of sorghum and Jerusalem artichoke biomass as fertilizers in the cultivation of energy crops. Seeds of *Sorghum bicolor* L. were placed in the Phytotoxkit-plates, filled with Alonet Substrate KS (Latvia) and containing 0-100% of ash from burnt sorghum and Jerusalem artichoke plants. Based on daily measurements under greenhouse conditions, dynamics of seed germination, growth speed of shoots and roots, their fresh and dry biomass, index of chlorophyll content and parameters of gas exchange were evaluated. The results indicated that the modified Phytotoxkit biotest could be useful for quick assessment of ashes usefulness as fertilizers, it can be performed under greenhouse conditions regardless of the growing season and may be an alternative to laborious and long-term field trials. Biotest showed that the studied ashes, used in the proper dosages, improved seed germination, plant growth and biomass yield. These events were associated with increased index of chlorophyll content in leaves, net photosynthesis, stomatal conductivity, transpiration and proportionally decreased concentration of intercellular CO_2. This indicate, that the studied ashes can be used as fertilizers in sorghum crops.

K e y w o r d s: ash, sorghum, Jerusalem artichoke, growth

INTRODUCTION

Management of ashes from burnt plant biomass and their use in plant cultivation as fertilizers are important issues in the world industry and agriculture. This problem has become serious because the burning of plant biomass is a perspective branch of energy production and an alternative to minerals whose combustion emits greenhouse gases that cause adverse climatic changes. The increasing amount of ashes from the combustion of plant biomass poses a serious problem of their economic and ecological management. Given the environmental benefits, it seems most rational to use them in agriculture as alternative to synthetic fertilizers. World literature available in this field is poor and in the majority of cases it treats the problem fragmentarily. Jagodziński *at al.* (2018) showed that wood ash beneficially influenced growth of *Lemna minor*. Santalla *et al*. (2011) and Vassiliev *at al*. (2010, 2013) indicated the possibility of using wood ash in forest plantation and in some other species cultivation. Ciesielczuk *et al.* (20011), Piekarczyk *at al*. (2011) and Meller and Bilenda (2012) demonstrated the beneficial effect of ashes, from burnt particular crops, on soil quality and the content of nutrients in it. According to our knowledge, the study on the possibility of using ashes from the burnt sorghum and Jerusalem artichoke in sorghum crops has not been conducted so far.

Quick and reliable assessment of ashes toxicity and of their effect on plant development that would allow to plan rational fertilization management is still a problem in agricultural application of this waste. Thus, plant physiologists are looking for methods that can quickly show plant reaction to used ashes. The obtained results are expected to help make quick decisions concerning fertilization without the need of labour-intensive and time-consuming field tests. One of such tests seems to be the modified Phytotoxkit biotest which performed in laboratory, irrespective of the season, can quickly point to the development and quality of plant shoots and roots under the influence of various fertilizers, as it was stated in the case of the assessment of soil and water contamination by pollutants (Grzesik and

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Romanowska-Duda, 2005; Romanowska-Duda *et al.,* 2006, 2010). It is presumed that this test may be part of a new crop monitoring strategy in which efforts should be made to discontinue or minimize the introduction of chemicals into the soil and plants. However, due to little information in this area, further research is necessary.

The aim of this research was to assess the suitability of the Phytotoxkit biotest for rapid assessment of usefulness of ashes, from burnt sorghum and Jerusalem artichoke biomass, as fertilizers in sorghum crop cultivation, which can be an alternative to the labour-intensive and time-consuming field tests.

MATERIALS AND METHODS

The sorghum (*Sorghum bicolor* L.) seeds of the Rona 1 variety were supplied by the breeding company 'Kutnowska Hodowla Buraka Cukrowego' in Poland and warehoused under standard storage conditions before use in experiments. In January, May and October the stored seeds were sown in modified by the authors Phytotoxkit plates (25 x 5 x 20 cm: length x width x depth), having glass front walls enabling assessment of root growth, and filled with 3 liters of sphagnum peat substrate (Alonet Substrate KS, Latvia). Two weeks before seed sowing, the ashes from burnt sorghum and Jerusalem artichoke shoots were sifted on sieves (with 2 x 2 mm mesh) and added to the Alonet Substrate KS in such amounts that their contents in these mixtures were 0, 1.2, 2.5, 5.0, 10, 15, 25, 50, 75 and 100% (v: v). The Phytotoxkit plates with the sown seeds were placed in a greenhouse, under conditions occurring in the studied months, and watered with tap water if necessary. In each Phytotoxkit plate 10 plants were cultivated and each treatment was performed in five replications. The plants cultivated in the substrate not containing ashes (0%), watered with tap water served as the control (Grzesik *et al*., 2017a).

Dynamics of seed germination, in each trial, was assessed by daily counting of the germinated seed number and performing calculations using the Seed Calculator Version 3.0 computer program developed by Plant Research International B.V., Wageningen, the Netherlands. Each seed was counted as having germinated when its radical protruded through the seed coat (Badek *et al*., 2014, 2016; Grzesik and Romanowska-Duda, 2014; Grzesik *et al*., 2017b; Górnik and Grzesik, 2002).

The length of roots and shoots was measured by a ruler from the germline up to their top parts, at 7-10-day intervals, depending on growth intensity. Fresh (freshly harvested) and dry biomass weights (after 3-day drying at 130°C in hot-air oven) of roots and shoots were evaluated at the end of experiment and calculated into a mass from one plant (Grzesik *et al*., 2017a).

The quality of roots was assessed in 1-5-degree scale at 3-15 day intervals, depending on intensity of plant growth. The 0-5-degree scale demonstrated that the root biomass filled 0-100% of the volume of Phytotoxkit plate, respectively.

The index of chlorophyll content in the leaves was evaluated during intensive plant growth using Minolta SPAD-502, Japan (Grzesik *et al*., 2017a,b). The gas exchange (net photosynthesis, transpiration, stomatal conductance and intercellular CO_2 concentration) was assessed using the gas analyzer apparatus TPS-2 (PP Systems, USA) (Grzesik and Romanowska-Duda, 2014, Grzesik *at al*., 2017 a,b; Kalaji *et al*., 2014).

The health of sorghum seeds was assessed by two methods: performing artificial cultures using selective nutrient media and a comparative paper test (TB) method. Plant health was assessed using moist cameras, in which fragments of plants were laid out and microbiological diagnostics was then carried out using light Leica microscopy.

Content of macro- and microelements in ash from the burnt sorghum and Jerusalem artichoke biomass and in sphagnum peat substrate was assessed in a certified laboratory of the Research Institute of Horticulture in Skierniewice.

All experiments were performed in three series and in five replicates for each treatment within each series. Within each series, each replicate was set up in a completely randomized block design. The obtained data, given as means from series and replicates, were processed applying analysis of variance (ANOVA), by Statistica 12. The means of chosen parameters of gas exchange and index of chlorophyll content were additionally grouped employing the Duncan's test at $\alpha = 0.05$ significance level.

RESULTS AND DISCUSSION

The obtained results demonstrate that the modified by the authors Phytotoxkit biotest, can be very useful in rapid assessment of the ash fertilization impact on sorghum seed germination and plant development and that it may be recommended for prediction of biomass production in field. This biotest is not expensive, not time-consuming and can be performed both under laboratory and greenhouse conditions, independently on season, as an alternative to laborious and long-term field studies which should be performed throughout the growing season. Because it is performed under specific conditions simulating the crop environment, it allows for making the right decisions regarding the treatment of plants during cultivation in the field and reduces the risk of their implementation. Due to the right amount of soil substrate, the plants can grow almost like those in the field and can be analysed in terms of the same assessment parameters, including biomass yield, content of nutrients and chlorophyll and gas exchange in the leaves. Moreover, the glass front wall allows to observe the dynamics of growth, quality and health of the roots, which are a plant organ that strongly reacts to the soil conditions. The obtained results refer to previous studies that demonstrated the usefulness of microbiotests, filled with filter paper instead of soil, for assessing the toxicity of pollutants in water using algae (Yan *et al.*, 2015), harmfulness of microcystins (Romanowska-Duda *et al.*, 2006), phytotoxicity of pharmaceuticals using horticulture and wild species (D'Abrosca *et al.*, 2008) suitability of sewage sludge as fertilizers (Romanowska *et al.*, 2010) or phytotoxicity of aqueous extracts of coal-contaminated soil (Radić *et al.,* 2018). However, these tests were not useful for simulating field conditions and assessing the growth and yield of plant biomass or their physiological activity.

The research performed in the modified Phytotoxkit plates demonstrated that ashes from the burnt sorghum and Jerusalem artichoke plants could be used, in the proper doses, as a fertilizer in *Sorghum bicolor* L. Moenh. crops since they have stimulatory impact on growth, biomass yield, physiological activity and positive influence on plant health. They contained a large quantity of macro- and microelements necessary for the plants development, especially lime rendering alkaline pH ranging from about 11 to 13, similarly as it was found in wood ash (Barbosa *at al*., 2013; Ciesielczuk *at al.*, 2011; Jagodziński *et al*., 2018; Römbke *et al*., 2009). The ashes did not contain nitrogen, which escapes into the atmosphere during the biomass combustion (Table 1). Weight of one unit of ash volume from the burnt sorghum was two times smaller than that of ash from Jerusalem artichoke. Although, the ashes show often large differences in their elemental composition depending on plant species (Schiemenz *et al.*, 2011), the composition of ash from burnt sorghum and Jerusalem artichoke is similar (Table 1).

Supplementation of the peat substrate with the studied ashes improved dynamics of seed germination and number of germinated seeds. This effect depended on the dose and the burnt plant species. The ash from the burnt sorghum, added to the substrate and constituting 5% of this mixture, was the most effective in improving germination dynamics and increasing the number of germinated seeds. Increasing the ash content up to 10 and more percent gradually reduced the germination rate and the number of germinated seeds leading to their near-disappearance at 100%. In the case of ash from burnt Jerusalem artichoke the found relationship was similar, although the optimal ash content in the substrate to improve germination was 2.5%. Increasing its content caused faster decrease in germination than in the case of sorghum (Fig. 1). These differences probably resulted from twice the lower weight of one unit of ash volume from burnt sorghum than from Jerusalem artichoke. The less weighing one unit of sorghum ash contained less nutrients and showed lower alkalization and toxicity than this from Jerusalem artichoke. Therefore, more ash from sorghum than from Jerusalem artichoke was necessary to obtain a stimulant effect on plants. The results are in line with Naghipour *et al*. (2016) research which showed that seed germination of *Bromus tomentellus* and *Bromus tectorum* was promoted by ash treatment, whereas the other studied species exhibited no response or were inhibited by these fire products.

Similarly as in the case of germination, the addition of ash from burnt sorghum biomass up to 5% of the content in peat substrate (v:v) and up to 2.5% from Jerusalem artichoke intensively increased and accelerated plant development which was exhibited by greater growth dynamics of shoots and roots. Increasing the amount of ash over the indicated content resulted in a gradual reduction in the growth of shoots and roots (Fig. 2). Simultaneously, during the entire growing period the used ashes did not enhance infestation of shoots and roots by pathogenic fungi, independently of used dosages and burnt plant species (data not show). The obtained results are consistent with the research of Puchalski *et al*. (2017), who showed a positive effect of ashes from the burnt coniferous biomass on the growth of two varieties of topinambour. The beneficial effect of ashes on sorghum growth could result from the increased contents of available potassium, phosphorus and magnesium in the soil, as Meller and Bilenda (2012) stated, investigating the effect of ashes from wood chips, energy willow, maize and

Table 1. Content of macro- and microelements in ash from the burnt sorghum and Jerusalem artichoke biomass and in Alonet Substrate KS (Latvia)

Assessed substrate	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn	B	Dry mass (%)
	mg kg^{-1} dry weight										
Ash from Jerusalem artichoke	0.40	8457	155200	143800	11690	2452	168	22.1	855	272	93.5
Ash from sorghum	0.48	14220	163000	38430	9961	2330	148	29.7	1148	61.9	89.6
Substrate KS*	mg l^{-1}										
	100 -200	120 -240	140 - 240	-	60 - 100	-	-	-	-	-	25.8

*pH (H_2O) 5.5 – 6.5, salinity 1.0 g l^{-1}.

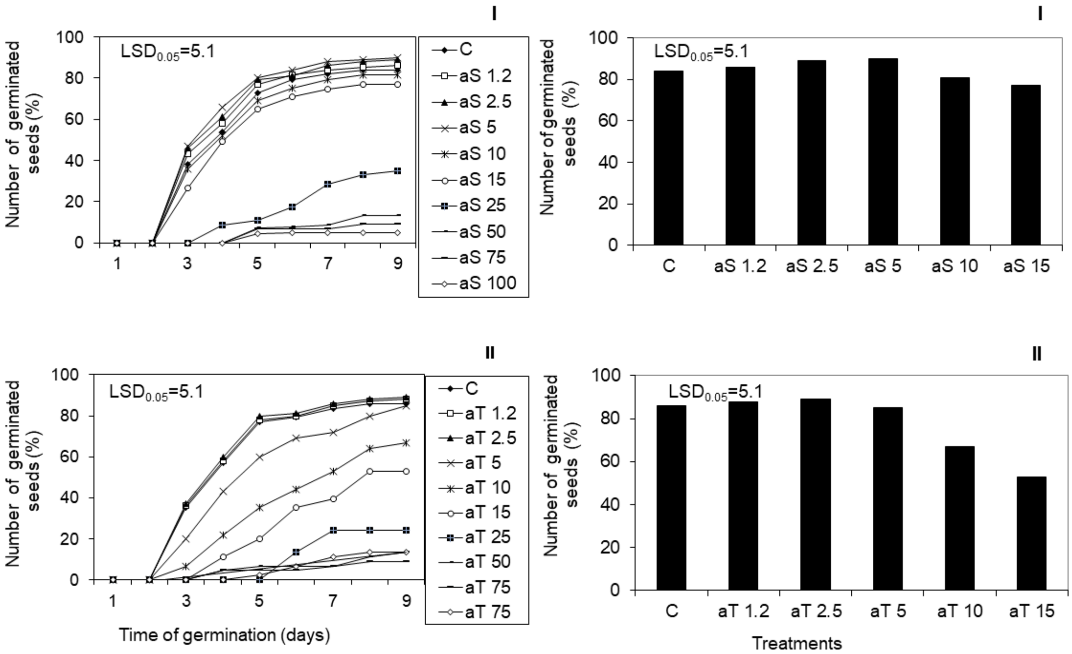

Fig. 1. Dynamics of sorghum seed germination and number of germinated seeds in the Phytotoxkit plates, filled with peat substrate containing 1.2 -100% (v:v) of ash from the burnt biomass of sorghum (I; aS) and Jerusalem artichoke (II; aT).

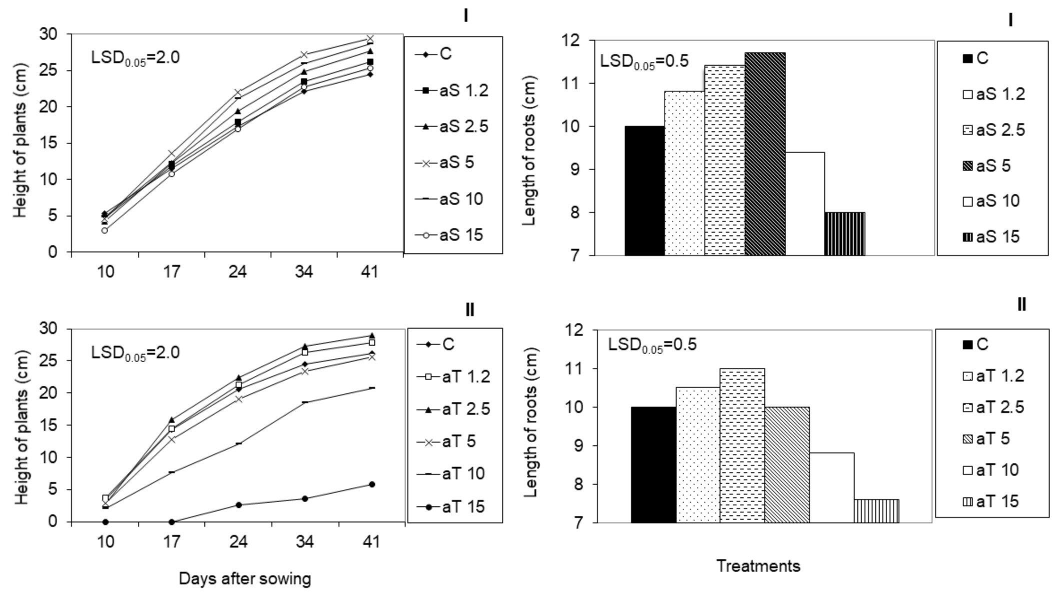

Fig. 2. Dynamics of shoot growth and root length of sorghum grown in the Phytotoxkit plates, filled with peat substrate containing 1.2 -15% (v;v) of ash from the burnt biomass of sorghum (I; aS) and Jerusalem artichoke (II; aT).

straw on sugar miscanthus growth. They showed that the amount of available potassium in the soil increased from medium to very high after fertilization with ash. According to Pitman (2009), Meller and Bilenda (2012), the effect of ash from biomass on the physicochemical properties of soils is comparable to that of mineral fertilizers. The application of ash increased the content of phyto-available forms of manganese and iron and other nutrients in soils to the level that is optimal for the proper development of plants.

The positive effect of ash, which were 5% (from burnt sorghum) and 2.5% (from burnt Jerusalem artichoke) of the mixture with the peat substrate (v;v), on seed germination resulted also in increased root quality, expressed in 1-5-degree scale. Under the influence of the mentioned ash quantities, the root system developed faster than in the control variant and when lower or higher doses were used. At the end of growth, the root biomass filled almost the entire space of the Phytotoxkit plates. All roots were also characterized by high quality, proper colour, shape and were not affected by pathogenic microflora (Fig. 3). The demonstrated high quality of the root system indicates the lack of toxicity of ashes from sorghum and Jerusalem artichoke in the optimal amounts used. Roots are one of the most sensitive plant organs reacting for unfavorable conditions and the chemical composition of the soil (Badek *et al*., 2014, 2016; Grzesik and Romanowska-Duda, 2005, 2014). The beneficial effect of used ashes on the root development indicates the legitimacy of these waste using as fertilizer in the studied plant cultivation.

The beneficial effect of ashes on seed germination and plant growth resulted also in their increased biomass yield. Similarly, as shown on the basis of previous biometric tests, the addition of ashes in the above mentioned optimal amounts to the peat substrate enhanced the yield of fresh and dry biomass of roots and shoots (Figs 4, 5). Similar results were obtained by Stankowski *et al*. (2014), who demonstrated the increased ear density and yield of wheat grains under the influence of ash fertilization from burnt plant biomass, used alone or jointly with compost.

Positive changes in the growth and yield of plant biomass were associated with their physiological activity. The used ashes positively influenced the index of chlorophyll content in the leaves and gas exchange assessed on the basis of net photosynthesis, transpiration, stomata conductance and intercellular CO_2 concentration. These parameters depended on the amount of applied ashes and burnt plant species, and the obtained relationships closely coincided with those observed in previous plant evaluation tests (Table 2), similarly as it was found in willow and apple plants obtained from differently treated cuttings and seeds (Grzesik *at al*., 2017a, b).

The obtained results indicate that ashes from the burnt sorghum and Jerusalem artichoke plant biomass can be recommended for fertilization of sorghum crops as a partial alternative to artificial fertilizers and the use of the Phytotoxkit test enables quick determination of their doses under specific cultivation conditions. The obtained results are in line with Schiemenz and Eichler-Löbermann (2010) research who showed that biomass ashes can be applied in agriculture as a valuable fertilizer, provided that they do not contain harmful concentrations of heavy metals or other toxic substances. According to them the crop biomass ashes can be an adequate P source comparable to that of highly soluble commercial P fertilizer. Additionally, the use of ashes on large scale in the plant production solves the ecological problems of their storage and will lower the pollution of environment (Insam and Knapp, 2011; Romanowska-Duda *et al*., 2006). The use of ash for crop fertilizing purposes enables a closed cycle of displacement of macro- and micronutrients, which return from the burned and not polluted biomass to the soil and then they are absorbed again by the plants. For this reason, because their content is constant, the soil environment does not degrade (Santalla *et al*., 2011; Vassiliew *et al*., 2013). However, the chemical composition of ashes from biomass may be varied in some cases and some of them may contain toxic elements (Vassiliew *et al*., 2010). According to Ciesielczuk *et al.* (2011), ashes may contain high levels of manganese, zinc and copper, depending on burnt plant species. This may decreasing their use as fertilizer. However, in the light of the applicable legal regulations, only ash obtained from pine wood should be eliminated from agricultural use due to the over-normative content of lead. Because the composition of ashes depends on various factors, their effect on plant development requires constant evaluation and therefore the quick biotest Phytotoxkit can be very useful here (Jagodziński *et al*., 2018). The use of elaborated biotest becomes necessary because data on both toxicity and plant growth promoting potential of ashes are scarce. The aquatic toxicological tests were used for assessing wood ash impact on some plant species (Barbosa *et al*., 2013). The accuracy of test assessments may depend on supply different-quantity levels of ashes and composition of medium which can mask stimulating effect of these fertilizers. The use of mediums containing low amount of nutrients allows to assess ashes stimulating properties more accuracy. For this reason in the presented modified Phytotoxkit plates the nutrient-poor substrate was used, being sure that the observed plant reactions to the used ashes will also appear

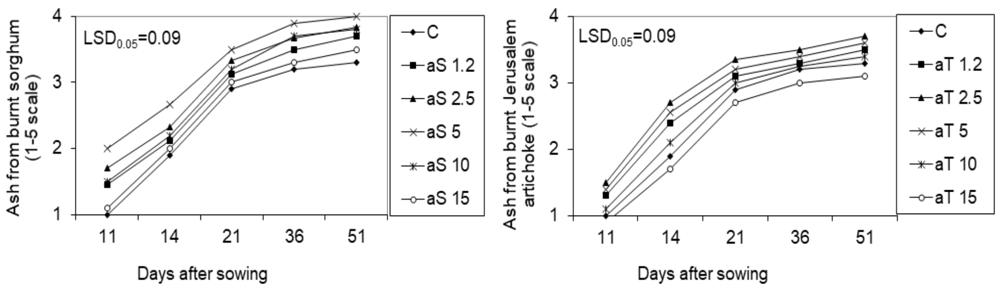

Fig. 3. The quality of roots (in 1-5-degree scale) of sorghum grown in the Phytotoxkit plates, filled with peat substrate containing 1.2 -15% (v:v) of ash from the burnt biomass of sorghum (aS) and Jerusalem artichoke (aT). The 0-5-degree scale demonstrates that the root biomass filled the volume of Phytotoxkit plate in 0-100%, respectively.

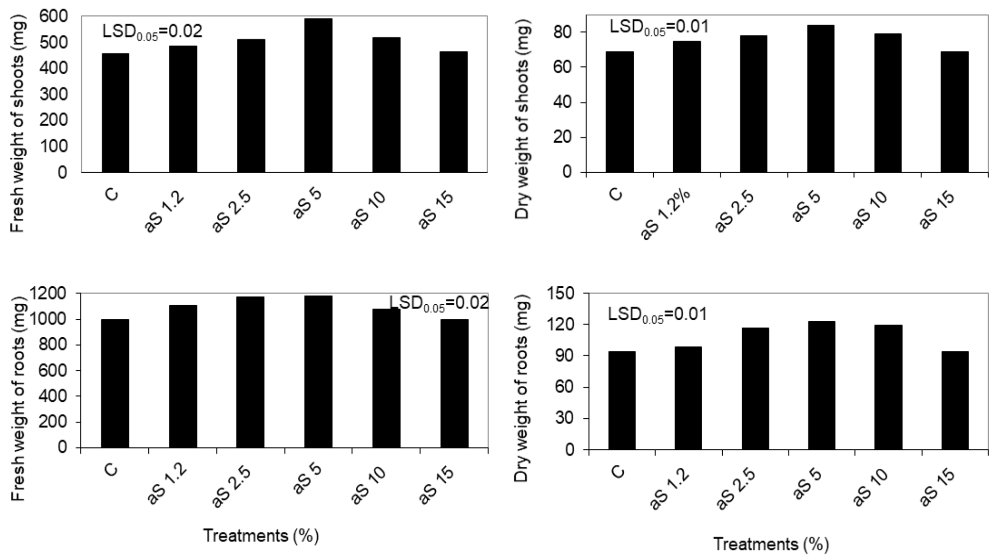

Fig. 4. Fresh and dry weight of shoot and roots of one sorghum plant grown in the Phytotoxkit plates filled with peat substrate containing 1.2-15% (v:v) of ash from the burnt sorghum biomass (aS).

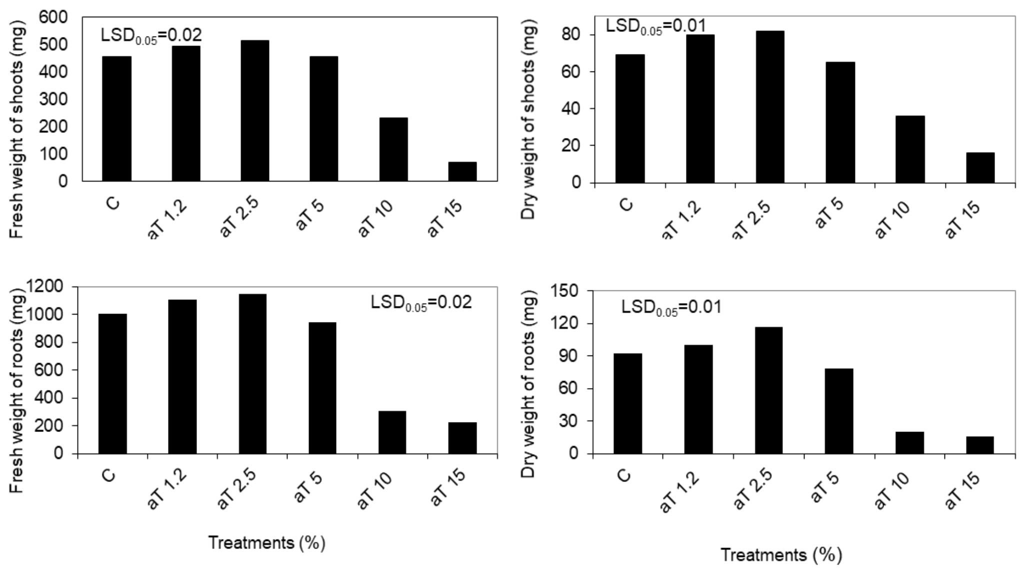

Fig. 5. Fresh and dry weight of shoot and roots in one sorghum plant grown in the Phytotoxkit plates filled with peat substrate containing 1.2-15% (v:v) of ash from the burnt Jerusalem artichoke biomass (aT).

in the field. Although, this effect in field may be less intensive and depend on both, the fertilizing effectiveness of ashes and masking properties of soil which can contain large amount of nutrients. The use of ashes for plant fertilizing poses also a problem concerning their high alkaline pH, sometimes 11-13% (Ciesielczuk *et al*., 2011). However, as the presented research show, this problem might not exist when the low recommended quantities of ashes in peat substrate and field soil are used, because of their capacitive sorbent complex, large volume, usually low pH and plant tolerance to this pH (Römbke *et al*., 2009). The potentials for the utilisation of biomass ash in land improvement for agriculture are yet to be fully explored. The use of biomass ash for soil improvement in forestry and farming involves a lot of indices and complex interactions that need to be well managed. Therefore, any application of biomass ash for soil improvement must be made on the basis of a long term flexible plans or programmes which shall include constant monitoring and testing (Ozuah, 2015).

The performed study indicate that the elaborated modified Phytotoxkit biotest can be useful to show the ash-fertilizing properties in sorghum crops. When it is performed

Table 2. Gas exchange and index of chlorophyll content in leaves of sorghum grown in the Phytotoxkit plates filled substrate containing 1.2-15% (v: v) of ash from the burnt sorghum (aS) and Jerusalem artichoke (aT) biomass

Applied ash	Net photosynthesis (μm CO_2 m^{-2} s^{-1})	Transpiration (mmol H_2O m^{-2} s^{-1})	Stomatal conductance (mmol m^{-2} s^{-1})	Intercellular concentration of CO_2 (μmol CO_2 air mol^{-1})	Index chlorophyll content (SPAD)
Control	4.94 a*	1.31 b	188.0 d	407.0 d	24.30 ab
Sorghum grown in peat and sand mixture containing 1.2-15% (v:v) of ash from burnt sorghum plants					
aS 1.2%	5.10 b	1.55 d	199.0 de	404.0 cd	26.50 c
aS 2.5%	5.90 d	1.85 f	215.0 e	386.0 b	28.56 d
aS 5%	6.30 e	1.84 f	217.0 e	372.0 a	28.73 g
aS 10%	5.40 c	1.59 d	200.0 de	398.0 c	24.83 b
aS 15%	5.35 c	1.43 c	130.0 b	420.0 e	24.45 ab
Sorghum grown in peat and sand mixture containing 1.2-15% (v:v) of ash from burnt Jerusalem artichoke plants					
aT 1.2%	5.00 ab	1.57 d	199.0 de	380.0 b	23.30 a
aT 2.5%	5.85 d	1.71 e	213.0 e	379.0 ab	27.97 d
aT 5%	5.45 c	1.49 c	165.0 c	420.0 e	26.56 c
aT 10%	5.00 ab	1.20 a	143.0 b	435.0 f	23.93 ab
aT 15%	4.90 a	1.15 a	80.0 a	440.0 f	23.32 a
$LSD_{0.05}$	0.11	0.10	20.2	7.8	1.2

*Data marked with the same letters, within column, are not significantly different, according to Duncan multiple range test at an alpha level of 0.05.

in a nutrient-poor medium it show effect on plants development only. However, when field soil is used, it allows to demonstrate the fertilizing effect of ashes under specific field conditions and the particular plant range. This makes it possible to make the right decisions regarding the fertilization of the scheduled crops with ash from selected burnt plant biomass. Therefore the use of developed Phytotoxkit test can reduce the risk of this fertilization implementation.

CONCLUSIONS

1. The modified Phytotoxic biotest is useful in rapid assessment of the ash suitability as fertilizers, it is not time-consuming and can be carried out under laboratory and greenhouse conditions independently of the season, as an alternative to laborious and long-term field studies.

2. Ash from the burnt sorghum and Jerusalem artichoke plants can be used as fertilizer in *Sorghum bicolor* L. Moenh. crops. It had stimulatory impact on seed germination, growth of plants, fresh and dry biomass yield of shoots and roots, physiological activity and it did not decrease plant health. However, this impact depended on its amounts applied to the substrate and the species of the burnt plant.

3. The best effects on germination of seeds, growth and physiological activity of sorghum plants were obtained when 5 and 2.5% of sorghum and Jerusalem artichoke ashes in the mixture with peat substrate were used, respectively.

4. Fertilization of crops with ashes from the burnt plant biomass can be a partial alternative to the use of artificial fertilizers, it is beneficial for the environment and the use of the Phytotoxkit test enables quick determination of their doses under specific cultivation conditions.

5. Soil fertilization with biomass ashes is consistent with the sustainable development and is a perspective way to dispose of them.

Conflict of interest: The Authors do not declare conflict of interest.

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