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Antioxidative activity of azoles and their influence on barley (*Hordeum vulgare* L.) seedlings and flavonoid accumulation

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Abstract

This research was aimed at exploring the antioxidative activity of new azole compounds and their influence on barley (*Hordeum vulgare* L.) growth *in vitro* and changes of flavonoid content. Azole derivative 5-{2-[(4-methylphenyl)amino]ethyl}-*N*-phenyl-1,3,4-thiadiazol-2-amine showed the best radical scavenging property. The highest growth regulating activity was demonstrated by 3-(4-methylphenyl)-1-phenyl-3-{2-[5-(phenylamino)-1,3,4-thiadiazol-2-yl]ethyl}thiourea. The length of the barley shoots, which were exposed to this compound (0.1 mg l^{-1}) increased by 42.7% in comparison with the control sample. Under its influence (0.4 mg l^{-1}) barley roots were by 72.3% longer than the ones of the control sample. The highest antioxidative activity was determined for the barley seedlings, which were exposed to the same *N*′-thiocarbamoyl-1,3,4-thiadiazole (0.1 mg l^{-1}).

Key words: biological activity, metabolites, oxadiazole, thiadiazole.

Introduction

Antioxidants are widely studied for their capacity to protect organisms and cells from damage induced by oxidative stress. New compounds, either synthesized or obtained from natural sources, attract attention of the scientists searching for the active components which could prevent or reduce the impact of oxidative stress on cells. Exogenous chemicals and endogenous metabolic processes in human body or in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules and thus causing cell death and tissue damage. Free radical oxidative processes also play a significant role in pathogenesis. Cancer, emphysema, cirrhosis, atherosclerosis, and arthritis have all been correlated with oxidative damage. In addition, excessive generation of reactive oxygen species induced by various stimuli leads to a variety of pathophysiological abnormalities such as inflammation, diabetes, genotoxicity, and cancer (Ünver et al., 2011).

The flavonoids are a remarkable group of plant metabolites. No other class of secondary product has been credited with so many or such diverse-key functions in plant growth and development. Flavonoids are known to enhance tolerance to a variety of abiotic stressors, they are employed as agents of defense against herbivores and pathogens, and they form the basis for allelopathic interactions with other plant species. The past decade has witnessed resurgence in research activity on the functions of flavonoids in plants because of their elevated capacity for scavenging free radicals associated with various diseases (Andersen, Markham, 2006). A large number of tests measuring their antioxidative activity *in vitro* have been reported (Nijveldt et al., 2001).

Barley (*Hordeum vulgare* L.) is an important crop both for brewing and for animal feed. In addition to conventional breeding, *in vitro* culture is a useful technology for the improvement of barley quality (Hall, 1999). The antioxidant properties of phenolic compounds, including flavonoids, in grains have been associated with the health benefits attributed to these crops and the value-added products derived from them. One of the richest sources of phenolics among the grains is barley. In beer, for example, 70% to 80% of the phenolic constituents originate from malted barley while the remaining 20% to 30% come from the hops (Gerhäuser, 2005). The scavenging activity of barley phenolics against DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) are comparable to a synthetic antioxidant BHT (butylated hydroxytoluene) (Ragaee et al., 2006). The flavone *C*-glycosides saponarin and luteorin have been found to be the major flavonoid antioxidants in young green barley leaves (Markham, Mitchell, 2003). The results of the investigation of the antioxidative and hypolipidemic effects of barley leaf essence in a rabbit model of atherosclerosis have suggested that this material can be utilised in the prevention of cardiovascular disease in which atherosclerosis is important (Yu et al., 2002).

Scientists in many different disciplines become more interested in new compounds, either synthesized or obtained from natural sources that could provide active components, possessing valuable properties. The

wide occurrence of the heterocycles in bioactive natural products, pharmaceuticals, and agrochemicals has made them important synthetic targets (Musad et al., 2011). Among different five-membered heterocyclic systems, derivatives of 1,3,4-thiadiazole and 1,3,4-oxadiazole have gained importance as they constitute the structural features of many bioactive compounds (Barbuceanu et al., 2012). Thiadiazole moiety acts as "hydrogen binding domain" and "two-electron donor system". It also acts as a constrained pharmacophore. Many drugs containing thiadiazole nucleus are available on the market such as acetazolamide, methazolamide, sulfamethazole, etc. Thiadiazole acts as the third and fourth generation cephalosporins, hence can be used in antibiotic preparations (Kushwaha et al., 2012). 1,3,4-oxadiazole/thiadiazole scaffold is an important pharmacophore in agricultural science and compounds bearing this moiety often display fungicidal (Xu et al., 2011), herbicidal (Jiang et al., 2010) and insecticidal (Luo, Yang, 2007) activities.

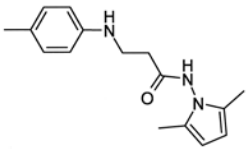
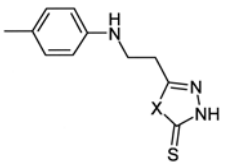
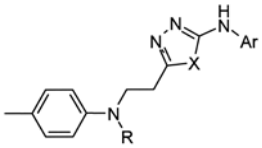
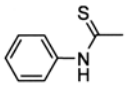
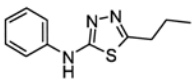
The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidative activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging has been thought to be due to their hydrogen donating ability (Madhu et al., 2011). The decrease in absorbance of DPPH radical is caused by antioxidants because of the reaction between antioxidant molecules and radical. This results in the scavenging of the radical by hydrogen donation.

The goal of this study was to explore bioactivity of new azole compounds, synthesized from *N*-(4-methylphenyl)-*β*-alanine hydrazide. These compounds were synthesized and investigated for the first time, therefore our objectives were: 1) to screen antioxidative activity of azole compounds, 2) to explore their influence on barley growth *in vitro*, 3) to investigate DPPH inhibition of barley plants, 4) to measure flavonoid amount changes in barley plants *in vitro*, 5) to recommend new azole compounds possessing promising biological activity for the future research *in vivo*.

Materials and methods

Azole derivatives, whose synthesis and characteristics have been described earlier (Tumosienė et al., 2012) were used for the tests grouped according to the structural similarities as listed in Table. Thiourea and thidiazuron (TDZ), the known growth regulators, were used in the tests as control treatments.

Table. Derivatives of pyrrole **A** and azolethiones **B** and **C**, and aminodiazoles **D**, **E**, **F**, **G** and **H** used for the tests

Number	Compound structure			Compound name
A				*N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-3-[(4-methylphenyl)amino]propanamide
				
	X			
B	O			5-{2-[(4-methylphenyl)amino]ethyl}-2,3-dihydro-1,3,4-oxadiazole-2-thione
C	NH_2N			4-amino-3-{2-[(4-methylphenyl)amino]ethyl}-4,5-dihydro-1*H*-1,2,4-triazole-5-thione
				
	X	R	Ar	
D	S	H	C_6H_5	5-{2-[(4-methylphenyl)amino]ethyl}-*N*-phenyl-1,3,4-thiadiazole-2-amine
E	S	H	4-$CH_3C_6H_4$	*N*-(4-methylphenyl)-5-{2-[(4-methylphenyl)amino]ethyl}-1,3,4-thiadiazole-2-amine
F	S		C_6H_5	3-(4-methylphenyl)-1-phenyl-3-{2-[5-(phenylamino)-1,3,4-thiadiazol-2-yl]ethyl}thiourea
G	S		C_6H_5	5-{2-[(4-methylphenyl)({2-[5-(phenylamino)-1,3,4-thiadiazol-2-yl]ethyl})amino]ethyl}-*N*-phenyl-1,3,4-thiadiazole-2-amine
H	O	H	3,5-$(CH_3)_2C_6H_3$	*N*-(3,5-dimethylphenyl)-5-{2-[(4-methylphenyl)amino]ethyl}-1,3,4-oxadiazole-2-amine

This research was carried out in 2011–2012 at Kaunas University of Technology, Faculty of Chemical Technology, Department of Organic Chemistry and Laboratory of Biotechnology. The seeds of barley cultivar 'Beatrix' (Saaten-Union GmbH, Germany) were used for the experiments. The seeds were initially washed in ethanol for 1 min; afterwards they were washed in bleach for 20 min and rinsed with distilled water. After sterilization, seeds were placed in 9-cm Petri dishes (total amount 150) on filter paper, which was wetted with 3 ml of distilled water (control sample) or solution of azole derivative. Azoles were examined at different levels of concentrations: 0.1, 0.2, 0.3, 0.4, and 0.5 mg l^{-1}. The results of experiments were compared with the ones of the control sample. Each Petri dish contained 10 seeds and was sealed with a strip of parafilm to prevent evaporation. Seeds were germinated in the dark for 7 days at room temperature (22°C) (Snow, Ghaly, 2008). Each experiment was repeated three times.

Biometric measurements. The length of axial organs of 30 selected barley seedlings per treatment was measured. In order to assess the tolerance of seedlings towards azoles we applied a Wilkinson tolerance index (WTI): $I_t = (l_{me}/l_c) \times 100\%$, where l_{me} indicates the increase in root growth in an azole solution and l_c is the increase in root growth in the control sample (Sazanova et al., 2012).

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay. Free radical scavenging activity of compounds was measured by DPPH using the widely used method (Madhu et al., 2011). Briefly, 1 mM solution of DPPH in ethanol was prepared, and this solution (1 ml) was added to the solutions of tested compounds (1 mg ml^{-1} of dimethyl sulfoxide). The mixture was shaken vigorously and allowed to stand at room temperature for 20 min. Afterwards, the absorbance was measured at 517 nm in a spectrophotometer UV-200-RS (MRC Ltd., Israel). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated according to the following equation: DPPH scavenging effect (%) = $(A_0 - A_1/A_0) \times 100$, where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the samples. The amount of flavonoids was determined according to $AlCl_3$ method (Farmakopėjos straipsnių rinkinys, 2001). A mixture of shredded plant material (2 g), acetone (20 ml), and 28% hydrochloric acid (2 ml) was heated under reflux for 30 min in a round-bottom flask. After cooling down, the hydrolizate was filtered off into a 100 ml volumetric flask. The remaining slurry was returned to the round-bottom flask, acetone (20 ml) was added and the mixture was heated under reflux for 10 min. After cooling down, the hydrolizate was filtered off into the same volumetric flask as the first part. The content of the flask was diluted with acetone up to 100 ml volume. The obtained solution (20 ml) was diluted with water (20 ml) and extracted with ethyl acetate: 1 × 15 ml and 3 × 10 ml. The combined upper fractions were washed with water (40 ml), filtered into 50 ml volumetric flask and the filtrate was diluted with ethyl acetate up to 50 ml volume. The test solution was prepared by adding 2 ml of $AlCl_3$ solution (20 g l^{-1}) to 10 ml of the main solution and filling the flask up to 25 ml volume by solution of acetic acid and methanol (1:19). The reference solution was prepared by adding the same acetic acid – methanol (1:19) solution to 10 ml of the main solution up to 25 ml volume. After 30 min, the absorbance was measured at 426 nm in a spectrophotometer UV-200-RS using the reference solution. The amount of flavonoids (X, %) was calculated as follows: $X = (A \times k) / m$, where A is the absorbance of the reference solution, k – a correction coefficient for hyperozide (k = 1.25), and m – mass of plant material (g). For the test of antioxidative capacity of barley extract, DPPH radical scavenging method was used. Dry barley seedlings (1.0 g) with 10.0 ml of methanol were homogenized for 10 min at room temperature. The homogenate was centrifuged at 10.000 g for 10 min and the supernatant was collected. Afterwards, the absorbance was measured at 517 nm in a spectrophotometer UV-200-RS. The antioxidative activity of the barley methanol extract against the DPPH radicals was expressed as follows: DPPH scavenging effect (%) = $(A_0 - A_1/A_0) \times 100$, where A_0 is the absorbance of the control reaction, and A_1 – the absorbance in the presence of the samples (Wang et al., 2007).

Statistical analysis. Differences between means were assessed by the *Student's t-test* at $P = 0.05$. Values were expressed as mean ± SD (Slapšytė et al., 2000).

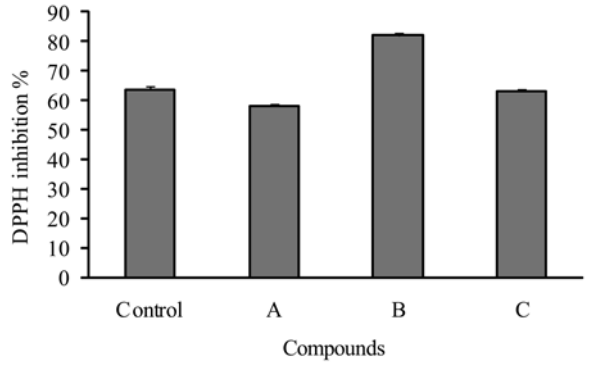
Results and discussion

Cressier has shown that 2-mercaptobenzothiazole and its 6-substituted derivatives display free radical scavenging activity towards DPPH (Cressier et al., 2009). *N*-(2,4-dimethylphenyl)-5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine (Khan et al., 2010) and 6-phenyl-3-(4-pyridyl)-1,2,4-triazolo-[3,4-b][1,3,4]thiadiazole (Cansiz et al., 2012) showed antioxidant activity. Musad synthesized and tested a series of bis(1,3,4-oxadiazole) derivatives as DPPH free radical scavengers (Musad et al., 2011). Derivative containing just phenyl ring had the best antioxidative capacity whereas the presence of either electron-donating or electron-withdrawing groups on the phenyl ring at positions 3,4,5 did not favour activity. Among the series of *N*-substituted phenyl-5-methyl-6-(5-(4-substituted phenyl)-1,3,4-oxadiazol-2-yl)thieno[2,3-d] pyrimidin-4-amines, the ones containing one or two fluorine atoms and chlorine atom showed significant radical scavenging activity (Kotaiah et al., 2012).

The data obtained in this study prove that the investigated compounds exhibit very good activity as radical scavengers, indicating that they have activity as hydrogen donors.

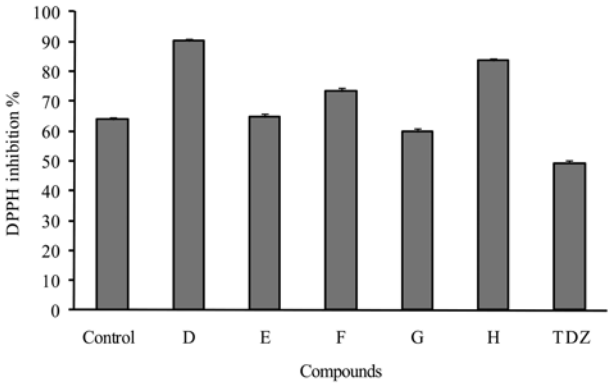
As seen from the results presented in Figure 1, in this group of the tested compounds, 1,3,4-oxadiazole-2-thione **B** showed the best radical scavenging property and its antioxidative activity was 1.28 times higher in comparison with that of thiourea. As shown in Figure 2, compounds **D**, **H** and **F** showed the highest radical scavenging properties. Antioxidative activity of 1,3,4-thiadiazole-2-amine **D** was 1.4 times higher in comparison with that of thiourea.

Based on the data provided in Figures 1 and 2, it can be concluded, that the highest antioxidative activity was demonstrated by thiadiazole derivative **D**, possessing phenyl ring at the NH group and no substituent at the nitrogen atom, which surpassed activity of oxadiazole derivative **H** and thiadiazole derivatives **F**, **E** and **G**, respectively, containing bulkier substituents at either of these positions. The treatment with 1,3,4-oxadiazole-



Compounds: **A** – *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-3-[(4-methylphenyl)amino]propanamide, **B** – 5-{2-[(4-methylphenyl) amino]ethyl}-2,3-dihydro-1,3,4-oxadiazole-2-thione, **C** – 4-amino-3-{2-[(4-methylphenyl)amino]ethyl}-4,5-dihydro-1*H*-1,2,4-triazole-5-thione

Figure 1. Antioxidative properties of the control (thiourea) and azoles **A**, **B** and **C**



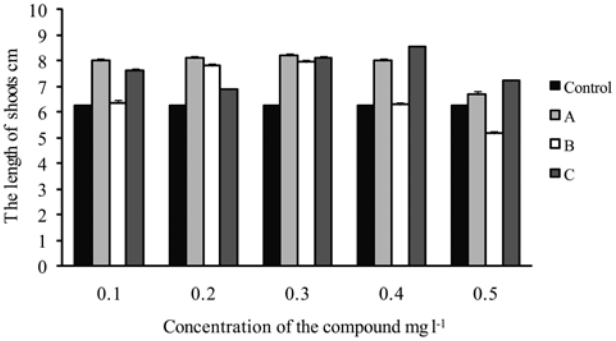
Compounds: **D** – 5-{2-[(4-methylphenyl)amino]ethyl}-*N*-phenyl-1,3,4-thiadiazole-2-amine, **E** – *N*-(4-methylphenyl)-5-{2-[(4-methylphenyl)amino]ethyl}-1,3,4-thiadiazole-2-amine, **F** – 3-(4-methylphenyl)-1-phenyl-3-{2-[5-(phenylamino)-1,3,4-thiadiazol-2-yl]ethyl}thiourea, **G** – 5-{2-[(4-methylphenyl) ({2-[5-(phenylamino)-1,3,4-thiadiazol-2-yl]ethyl})amino] ethyl}-*N*-phenyl-1,3,4-thiadiazole-2-amine, **H** – *N*-(3,5-dimethylphenyl)-5-{2-[(4-methylphenyl)amino]ethyl}-1,3,4-oxadiazole-2-amine

Figure 2. Antioxidative properties of the control (thiourea), azoles **D**, **E**, **F**, **G** and **H**, and thidiazuron (TDZ)

2-thione **B** gave better results in comparison with thiourea, whereas the effect of 1,2,4-triazole-5-thione **C** was worse than that of thiourea. It should be noted that, in all cases, the radical scavenging property of the synthesized compounds was better than that of TDZ.

Effects of azoles on length of barley shoots in vitro. In this experiment, the influence on the growth parameters of barley plants *in vitro* was investigated. Thiazole derivatives increase vegetative growth of plants, flowering, rooting of cuttings, inhibit falling of fruits, accelerate germination of seeds, stimulate efflorescence of buds, increase biosynthesis of ethylene, increase yield of rice (*Oryza sativa* L.), tomatoes (*Lycopersicon* Mill.), soybean (*Glycine* Willd), and grapes (*Vitis* L.). Triazole derivatives stimulate germination of common flax (*Linum usitatissimum* L.) and regulate growth of tomatoes, oats (*Avena* L.), sugar beet (*Beta vulgaris* L.), and grass. Besides, 1,3,4-thiadiazole-2-amine derivatives are used in mixtures with growth regulators and fertilizers (Икрина, Колбин, 2004).

In the first group of the tested compounds (Fig. 3), the length of barley shoots was the highest when barley seeds were treated with 1,2,4-triazole-5-thione **C** (0.4 mg l^{-1}). The barley shoots grew longer by 2.3 cm (37%) in comparison with the control sample. Under the influence of 0.5 mg l^{-1} concentration solution of 1,3,4-oxadiazole-2-thione **B** barley shoots were shorter by 82.8% in comparison with the control sample. When seeds were treated with 0.1, 0.2, and 0.3 mg l^{-1} solutions of the tested compounds, the best results were obtained for pyrrole derivative **A** (28.21, 29.8, and 31.41 %, respectively).

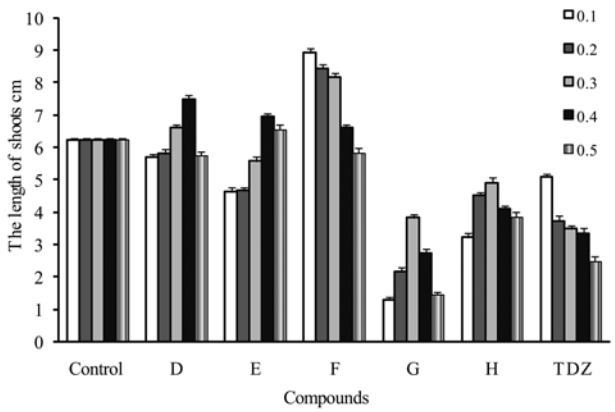


Explanations under Figure 1

Figure 3. The influence of the control (water) and compounds **A**, **B** and **C** on length of barley shoots *in vitro*

In the second group of the tested compounds (Fig. 4), the longest barley shoots were obtained when the seeds were treated with *N'*-thiocarbamoyl-1,3,4-thiadiazole **F** (0.1 mg l^{-1}). This compound had positive influence in all concentrations, except 0.5 mg l^{-1}. Length of barley shoots, when seeds were treated with compound **F** (0.1 mg l^{-1}), was by 2.4 cm (42.7%) longer in comparison with the control sample. Thiadiazole derivative **D** containing the phenyl substituent at the NH group had the highest effect, when barley seeds were exposed to 0.4 mg l^{-1} concentration and increased the length of shoots by 1.24 cm (19.8%) in comparison with the control sample. Under the influence of compounds **G**, **H**, and TDZ, barley shoots grew shorter by 23.24, 61.7, and 40.06 %, respectively (at 0.5 mg l^{-1} concentration), in comparison with the control sample.

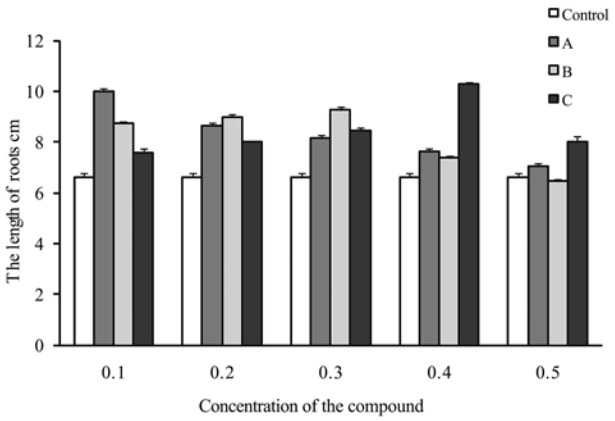
In conclusion, the results presented in Figures 3 and 4 had demonstrated, that the length of barley shoots *in vitro* was the most influenced by compound **F** containing phenylthioureido moiety. Slightly worse results were obtained under the influence of pyrrole derivative **A**, 1,2,4-triazole-5-thione **C**, and 1,3,4-triazole-5-thione **B**, respectively. 1,3,4-thiadiazole-2-amines **E** and **D**, containing no substituent at the nitrogen atom, gave better results than 1,3,4-oxadiazole-2-amine **H** which has no substituent at the nitrogen atom as well.



Explanations under Figure 2

Figure 4. The influence of the control (water), compounds **D**, **E**, **F**, **G** and **H**, and thidiazuron (TDZ) on length of barley shoots *in vitro*

As the results provided in Figure 5 indicate, among the first group compounds, 1,2,4-triazole-5-thione **C** stimulated the most growth of barley roots (0.4 mg l^{-1}) in comparison with the control sample. This compound increased the length of barley roots by 3.7 cm (55.2%) in comparison with the control sample.

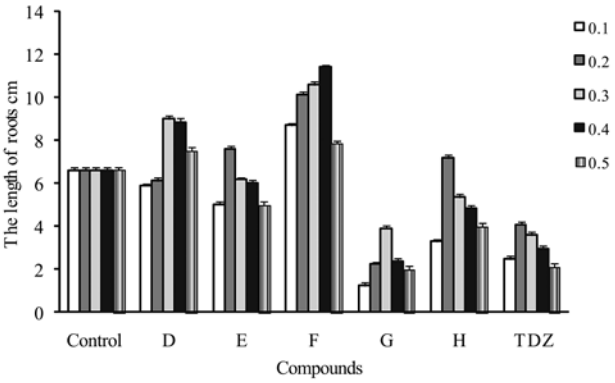


Explanations under Figure 1

Figure 5. The influence of the control (water) and compounds **A**, **B** and **C** on length of barley roots

Among the second group compounds (Fig. 6), barley roots grew the longest when seeds were treated with *N'*-thiocarbamoyl-1,3,4-thiadiazole **F** (0.4 mg l^{-1}). It increased the length of barley roots by 72.3% and they were 4.8 cm longer in comparison with the control sample. The second best compound was 1,3,4-thiadiazole-2-amine **D** containing no substituent at the nitrogen atom. Under the influence of compounds **E**, **G**, **H**, and TDZ barley shoots grew shorter by 75.64, 30.25, 60.51, and 31.77 %, respectively (at 0.5 mg l^{-1} concentration), in comparison with the control sample.

The dependence between the structure and biological activity analysis of the data presented in Figures 3, 4, 5, and 6 indicated, that the highest growth stimulating effect has been shown by compound **F**, containing 1,3,4-thiadiazole ring and phenyl substituent in it as well as phenylthiocarbamoyl substituent at the nitrogen atom. Interestingly, thiadiazole derivative **G** gave the worse results, though its structure differed from the one of compound **F** by the substituent at the nitrogen atom. If the activity of compounds **B** and **C** is compared, the effect of triazole-2-thione derivative **C** was higher than that of oxadiazole-2-thione **B**.

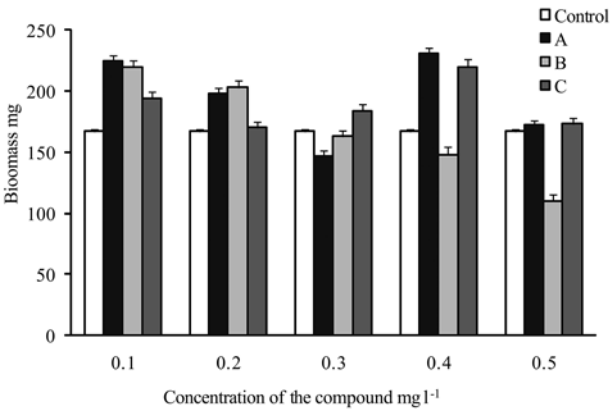


Explanations under Figure 2

Figure 6. The influence of the control (water), compounds **D**, **E**, **F**, **G** and **H**, and thidiazuron (TDZ) on length of barley roots

The influence of azoles on biomass of barley seedlings in vitro. The task in this part of the work was to explore the influence of azoles on biomass of barley seedlings *in vitro*. Triazole substituted oxathiolane compounds (Friedlander et al., 1986) and 2-(4-biphenylyl)-1-(2,4-dichlorophenyl)- and -phenyl-3-(1,2,4-triazol-1-yl)-2-propanol and 4-biphenylyl-2-chloro(fluoro)-phenyl-(1,2,4-triazol-1-yl-methyl)-carbinol have good plant growth regulating properties (Holmwood et al., 1992).

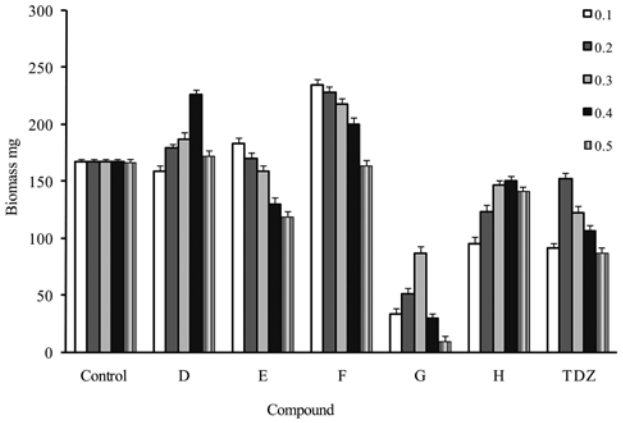
The investigation revealed (Fig. 7) that among the first group compounds, the biomass of barley seedlings was the biggest when seeds were treated with pyrrole derivative **A** (0.4 mg l^{-1}). The biomass was bigger by 64 mg (38.3%) in comparison with the control sample.



Explanations under Figure 1

Figure 7. The influence of the control (water) and compounds **A**, **B** and **C** on biomass of barley seedlings *in vitro*

As seen from the results of the second group compounds presented in Figure 8, the biomass of barley seedlings was the biggest when seeds were exposed to *N′*-thiocarbamoyl-1,3,4-thiadiazole **F** (0.1 mg l^{-1}). The biomass was bigger by 67 mg (40.1%) in comparison with the control sample. Thiadiazole **D** gave the second best results at 0.4 mg l^{-1} concentration, whereas compounds TDZ, **H**, and **G** decreased (52.1, 84.43, and 5.99 %, respectively) the biomass of the barley seedlings.



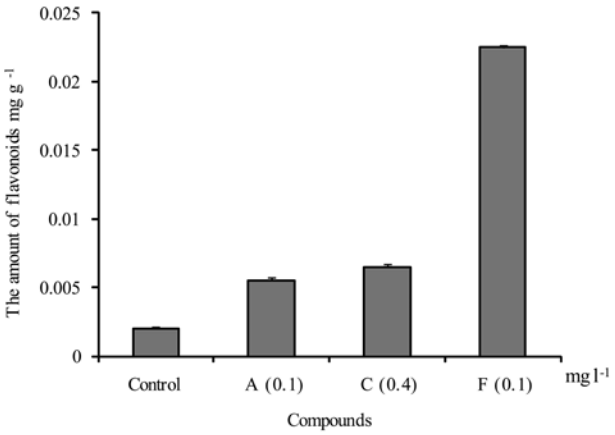
Explanations under Figure 2

Figure 8. The influence of the control (water), compounds **D**, **E**, **F**, **G** and **H**, and thidiazuron (TDZ) on biomass of barley seedlings *in vitro*

Based on the data provided in Figures 7 and 8 it can be concluded, that the biggest increase of the biomass of barley seedlings was achieved under the influence of *N′*-thiocarbamoyl-1,3,4-thiadiazole **F** which was followed by pyrrole derivative **A**. Thiadiazole derivative **D**, whose structure differed from the one of compound **F** by the absence of the substituent at the nitrogen atom, showed the third best result. As it was observed in the cases described above, thiadiazole derivatives were more active than the oxadiazole derivative except compound **G** which gave the most negative result surpassed just by TDZ. The effect of triazole-2-thione derivative **C** was higher than that of oxadiazole-2-thione **B** as well.

The amount of flavonoids and DPPH inhibition. Some studies have revealed that flavonoids inhibit lipid peroxidation and low-density-lipoprotein (LDL) oxidation (Sanchez-Moreno et al., 2000; Sang et al., 2003). Moreover, *in vitro* experimental data also suggest that flavonoids possess antiinflammatory, antiallergic, antiviral, and anticarcinogenic properties (Nijveldt et al., 2001). The amount of flavonoids and DPPH inhibition was determined in barley seedlings, which were treated with the most active compounds **A** (0.1 mg l^{-1}), **C** (0.4 mg l^{-1}) and **F** (0.1 mg l^{-1}).

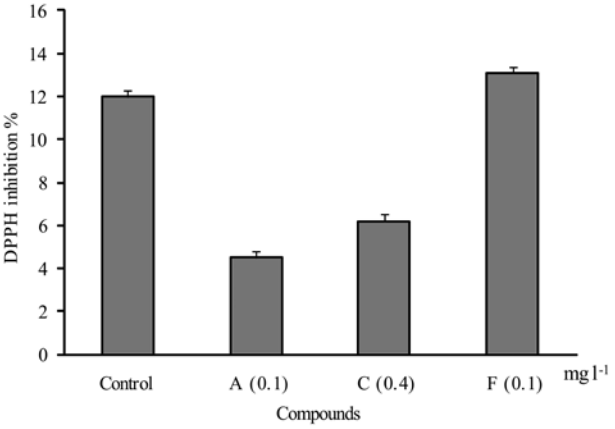
Results showed (Fig. 9) that the highest amount of flavonoids was in barley seedlings *in vitro*, which were treated with *N′*-thiocarbamoyl-1,3,4-thiadiazole **F** (0.1 mg l^{-1}). This compound increased the amount of flavonoids 11.25 times in comparison with the control.



Compounds: **A** – *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-3-[(4-methylphenyl)amino]propanamide, **C** – 4-amino-3-{2-[(4-methylphenyl)amino]ethyl}-4,5-dihydro-1*H*-1,2,4-triazole-5-thione, **F** – 3-(4-methylphenyl)-1-phenyl-3-{2-[5-(phenylamino)-1,3,4-thiadiazol-2-yl]ethyl}thiourea.

Figure 9. The amount of flavonoids in barley seedlings

Under the influence of the same *N′*-thiocarbamoyl-1,3,4-thiadiazole **F** (0.1 mg l^{-1}) the highest antioxidative activity was achieved for the barley seedlings (Fig. 10). This compound increased antioxidative activity of barley seedlings 1.1 times in comparison with the control sample.



Explanations under Figure 9

Figure 10. Antioxidative activity of barley seedlings

Conclusions

1. It was determined that among the tested azole derivatives, compounds containing 1,3,4-thiadiazole moiety were the most active ones. Biological activity of the majority of the tested azole derivatives was better than that of thidiazuron (TDZ).

2. 5-{2-[(4-methylphenyl)amino]ethyl}-*N*-phenyl-1,3,4-thiadiazole-2-amine (**D**) was found to be significant scavenger of free radicals.

3. 3-(4-methylphenyl)-1-phenyl-3-{2-[5-(phenylamino)-1,3,4-thiadiazol-2-yl]ethyl}thiourea (**F**), i.e. compound, whose structure differed from that

of compound **D** by phenylthiocarbamoyl substituent in the amino group, was identified as the best growth stimulator.

4. The highest amount of flavonoids was accumulated under the influence of the same 3-(4-methylphenyl)-1-phenyl-3-{2-[5-(phenylamino)-1,3,4-thiadiazol-2-yl]ethyl}thiourea (**F**).

5. Thiadiazole derivatives **D** and **F** are recommended as promising growth regulating agents for the future research in the mixtures with fertilizers under field conditions.

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Azolų antioksidacinis aktyvumas ir jų įtaka paprastojo miežio (*Hordeum vulgare* L.) daigams bei flavonoidų kaupimui

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Santrauka

Tyrimo tikslas – ištirti azolų antioksidacinį aktyvumą ir jų įtaką miežių augimui *in vitro* bei flavonoidų kiekiui. Iš tirtų junginių didžiausiu antioksidaciniu aktyvumu pasižymėjo 5-{2-[(4-metilfenil)amino]etil}-*N*-fenil-1,3,4-tiadiazol-2-aminas. Nustatyta, kad didžiausią miežių augimą skatinantį poveikį turėjo 3-(4-metilfenil)-1-fenil-3-{2-[5-(fenilamino)-1,3,4-tiadiazol-2-il]etil}tiokarbamidas. Šio junginio tirpale (0,1 mg l^{-1}) auginti miežių ūgliai buvo 42,7 % aukštesni, o miežių, apdorotų šio junginio 0,4 mg l^{-1} koncentracijos tirpalu, šaknys buvo 72,3 % ilgesnės, lyginant su kontroliniu variantu. Didžiausias antioksidacinis daigų aktyvumas pasireiškė miežiuose, augintuose 3-(4-metilfenil)-1-fenil-3-{2-[5-(fenilamino)-1,3,4-tiadiazol-2-il]etil}tiokarbamido tirpale (0,1 mg l^{-1}). Dauguma tirtų junginių buvo aktyvesni nei žinomas augimo reguliatorius tidiazuronas (TDZ).

Reikšminiai žodžiai: biologinis aktyvumas, metabolitai, oksadiazolas, tiadiazolas.