Synthesis of 1-propargyl-1-(2-methyl-3-oxo-3-(*p*-tolyl)propyl)-piperidin-1-ium bromide and its plant growth-stimulating activity assessment

Y.O. Belyankova[1]*, A.A. Dauletbakov[1,2], B.B. Anapiyayev[2], A.Yu. Ten[1,3], M. Aydemir[4], A.G. Zazybin[1,2]

[1]Kazakh-British Technical University, Department of Chemical Engineering, Almaty, Kazakhstan
[2]School of Chemical & Biochemical Engineering, Satbayev University, Almaty, Kazakhstan
[3]JCS "A.B. Bekturov Institute of Chemical Sciences", Almaty, Kazakhstan
[4]Dicle University, Diyarbakir, Turkey
*E-mail: *belyankovae@gmail.com*

The aim of this study was to synthesize novel ionic compound – 1-propargyl-1-(2-methyl-3-oxo-3-(*p*-tolyl)propyl)-piperidin-1-ium bromide – via the *N*-alkylation (in conventional conditions and using ultrasound activation), and investigate its influence on the plant growth-stimulating activity using of sweet sorghum seeds. The synthesized compound was fully characterized by infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy. Regardless of the type of synthesis' methods, the isolated yield of the produced ionic compound is showed 79-81%, however, the reaction rate was significantly increased by using ultrasonic activation. For the assessment of the growth-stimulating activity of the synthesized ionic compound, parameters such as root length, shoot length, seed energy germination rate, and germination capacity were determined on 10 genotypes of sweet sorghum seeds. The results of samples with low concentration (0.001% vol. solution) were demonstrated the higher intensity for tested genotypes than control samples, especially for process of gemmogenesis and intensity of rhizogenesis. The results of this study can be used as basis for the further development of plant growth stimulants based on ionic compounds.

Keywords: tolperisone; ionic compound; ultrasound activation; sweet sorghum; germination; plant growth-stimulating activity.

1-Пропаргил-1-(2-метил-3-оксо-3-(п-толил)пропил)-пиперидин-1-иум бромидінің синтезі және оның өсімдіктердің өсу-белсенділігіне әсерін бағалау

Е.О. Белянкова[1]*, А.А. Даулетбаков[1,2], Б.Б. Анапияев[2], А.Ю. Тен[1,3], М. Айдемир[4], А.Г. Зазыбин[1,2]

[1]Қазақстан-Британ техникалық университеті, "Химиялық инженерия" ғылыми-білім беру орталығы, Алматы қ., Қазақстан
[2]Сәтбаев университеті, Химиялық және биологиялық технологиялар институты, Алматы қ., Қазақстан
[3]АҚ «А.Б. Бектұров атындағы Химия ғылымдары институты», Алматы қ., Қазақстан
[4]Дикле университеті, Диярбакыр қ., Түркия
*E-mail: *belyankovae@gmail.com*

Берілген зерттеудің мақсаты жаңа иондық қосылыс - 1-пропаргил-1-(2-метил-3-оксо-3-(п-толил)пропил)-пиперидин-1-иум бромидті, *N*-алкилдеу арқылы синтездеу (қалыпты жағдайда және ультрадыбыстық белсендірумен) және тәтті жүгерінің дәндерін қолданып, оның өсімдіктердің өсу ынталандыру белсенділігіе әсерін зерттеу. Алынған қосылыс инфрақызыл спектроскопиясы (ИҚ) және ядролық-магнитті резонанс спектроскопиясы (ЯМР) арқылы сипатталынды. Алынған қосылыстың шығымы синтез әдісіне байланысты емес - 78-81%, бірақ қалаулы өнімнің құрылуы ультрадыбыстық белсендірумен әлдеқайда тезірек болады. Синтезделген иондық қосылыстың өсу ынталандыру белсенділігін тексеру кезінде тамыр ұзындығы, жапырақ ұзындығы, өну энергиясы және зертханалық өнгіштік сияқты параметрлер тәтті жүгері дәндерінің 10 генотипында өлшелінді. Қосылыстың ең кішкентай концентрациядағы ерітіндісі (0,001%) жақсы көрсеткіштер көрсетеді, әсіресе тамыр және жапырақ дамуының процессіне. Осы зерттеудің нәтижелері келесі әлеуетті экологиялық-қауіпсіз өсімдік өсу ынталандырғыштар үшін негіз ретінде қолданыла алады.

Түйін сөздер: толперизон; иондық қосылыс; ультрадыбыспен белсендіру; тәтті құмай; өну; өсуді ынталандыратын белсенділік.

Синтез 1-пропаргил-1-(2-метил-3-оксо-3-(*п*-толил)пропил)-пиперидин-1-иум бромида и оценка его ростстимулирующей активности

Е.О. Белянкова[1]*, А.А. Даулетбаков[1,2], Б.Б. Анапияев[2], А.Ю. Тен[1,3], М. Айдемир[4], А.Г. Зазыбин[1,2]

[1]Казахстанско-Британский технический университет, Научно-образовательный центр «Химическая инженерия», г. Алматы, Казахстан
[2]Университет Сатбаева, Институт химических и биологических технологий, г. Алматы, Казахстан
[3]АО «Институт химических наук имени А.Б. Бектурова», г. Алматы, Казахстан
[4]Университет Дикле, г. Диярбакыр, Турция
*E-mail: *belyankovae@gmail.com*

Целью данного исследования было синтезировать новое ионное соединение –1-пропаргил-1-(2-метил-3-оксо-3-(п-толил)пропил)-пиперидин-1-иум бромид – с помощью N-алкилирования (в обычных условиях и с использованием ультразвуковой активации) и исследовать его влияние на ростостимулирующую активность растений с использованием семян сладкого сорго. Полученное соединение было охарактеризовано с помощью инфракрасной (ИК) спектроскопии и спектроскопии ядерно-магнитного резонанса (ЯМР). Выход полученного соединения составил 79–81% вне зависимости от метода синтеза, однако образование желаемого соединения происходит значительно быстрее при ультразвуковой активации. Для проверка ростостимулирующей активности синтезированного ионного соединения были измерены такие параметры как длина корня, длина листа, энергия прорастания и лабораторная всхожесть на 10 генотипах семян сладкого сорго. Раствор вещества с меньшей концентрацией (0,001%) показывает хорошие показатели, в особенно на процесс развития корней и листьев. Результаты этого исследования могут послужить базой для дальнейшей разработки стимуляторов роста растений на основе ионных соединений.

Ключевые слова: толперизон; ионное соединение; активация ультразвуком; сладкое сорго; прорастание; ростстимулирующая активность.

(2022) Chem Bull Kaz Nat Univ 2:20-25

CHEMICAL BULLETIN

of Kazakh National University

http://bulletin.chemistry.kz/

UDC 54.057

https://doi.org/10.15328/cb1259

Synthesis of 1-propargyl-1-(2-methyl-3-oxo-3-(*p*-tolyl)propyl)-piperidin-1-ium bromide and its plant growth-stimulating activity assessment

Y.O. Belyankova[1]*, A.A. Dauletbakov[1,2], B.B. Anapiyayev[2], A.Yu. Ten[1,3], M. Aydemir[4], A.G. Zazybin[1,2]

[1]Kazakh-British Technical University, Department of Chemical Engineering, Tole bi str. 59, 050000 Almaty, Kazakhstan
[2]School of Chemical & Biochemical Engineering, Satbayev University, Satpaev str. 22a, 050013 Almaty, Kazakhstan
[3]JCS "A.B. Bekturov Institute of Chemical Sciences", Sh. Ualikhanov str. 106, 050010 Almaty, Kazakhstan
[4]Department of Chemistry, Faculty of Science, Dicle University, 21280, Diyarbakir, Turkey
*E-mail: *belyankovae@gmail.com*

1. Introduction

Sorghum (*Sorghum bicolor (L.)*) is a very important gain crop, it could produce a large yield and adapted under rough conditions such as heat and drought [1]. A substantial variation of sugar content makes sweet sorghum a potential source of biofuel [1]. It is used for animal feed [2], alcohol [1,4], syrups, glucose, modified starches, citric acid, etc. [3,5]. To stimulate the germination of sorghum different growth regulators are recommended [6]. Recently, *N*-methyl derivative of tolperisone was found practically harmless toward A. Fischeri [7], which makes other derivatives of tolperisone potentially promising environmentally friendly growth stimulants. In the present study, we describe the synthesis of a new ionic compound obtained via *N*-alkylation of tolperisone and its germination stimulating activity.

2. Experiment

2.1 Materials and research methods. Synthesis of 1-propargyl-1-(2-methyl-3-oxo-3-(*p*-tolyl)propyl)-piperidin-1-ium bromide

The melting point of ionic compound was measured in open capillary tube on an OptiMelt (Stanford Research System). IR spectra were recorded on Nicolet «5 700 FT-IR» spectrometer in a KBr tablet. ^{13}C NMR and ^{1}H spectra of synthesized compounds were recorded on «JNM-ECA Jeol 400» (100.53 and 399.78 MHz respectively) using $CDCl_3$ as solvent, the shooting temperature is 25°C. Control over the process of reactions and individuality of compounds was carried out by TLC on SiO_2 plates (Sigma Aldrich®, Germany) with mixture of diethyl ether:ethanol (4:1) as eluent and with iodine vapors development. Tolperisone freebase was obtained from Mydocalm® for synthesis at the room temperature and from corresponding hydrochloride (Shanghai Acmec Biochemical Technology Co, Ltd.) for synthesis with ultrasound activation. All reactants and solvents from Sigma Aldrich®. An ultrasonic probe from Cole Parmer (50-60Hz, 0-240 W) was used for the reaction. The separation and purification of the substances was carried out by recrystallization from appropriate solvents.

"Room temperature" method: In 15 ml acetonitrile, which contains 7.3 mmol tolperisone free-base, 5 ml of new acetonitrile with 8.03 mmol propargyl bromide was added. After mixing the reaction was carried in room temperature for 6 days. The precipitate was washed by cold acetonitrile and purified by recrystallization from diethyl ether: ethanol = 1:4. Product is beige crystals. Yield 81%. mp = 180°C. IR (KBr), cm^{-1}: 1670 (C=O), 1132 (C-N),3264 (≡C-H)

"Ultrasound activation" method: Tolperisone free-base (9.5 mmol) was dissolve in 15 ml acetonitrile and propargyl bromide (11.1 mmol) was added. Reaction was carried out with ultrasound activation (240 W, 30–50°C) for 5 hours, after precipitate was formed, it was washed by cold acetonitrile and purified by recrystallization from diethyl ether: ethanol = 2:3. Product is beige crystals. Yield 79%. mp = 177°C. IR (KBr), cm^{-1}: 1670 (C=O), 1132 (C-N), 3264 (≡C-H)

^{1}H NMR ($CDCl_3$) δ, ppm: 1.35 – 1.40 (C_{12}), 1.64 – 2.04 ($C_{2,3,4,9}$), 2.36 – 2.39 (C_{21}), 3.37 – 3.45 ($C_{1ax,5ax}$), 3.57 – 3.68 ($C_{1eq,5eq}$), 3.89 – 3.99 (C_{10ax}), 4.01 – 4.10 (C_{10eq}), 4.56 – 4.63 (C_{11}), 5.10 – 5.16 (C_7), 7.24 – 7.33 ($C_{17,19}$), 8.14 – 8.18 ($C_{16,20}$). ^{13}C NMR ($CDCl_3$) δ, ppm: 19.99 (C_{12}), 20.08 ($C_{2,4}$), 20.62 (C_3), 21.87 (C_{21}), 35,65 (C_{11}), 51.67 (C_7), 60.35 (C_{10}), 60.87 ($C_{1,5}$), 71.84 (C_8), 81.67 (C_9), 129.77 ($C_{17,19}$), 131.36 ($C_{16,20}$), 131.36 (C_{18}), 145.50 (C_{15}), 199.96 (C_{13}).

2.2 Assessment of plant grow-stimulating activity

The synthesized ionic compound was tested for germination energy and capacity with 10 sorghum genotypes (*KAZ-16 2013, Victoria-4 2013, Victoria-4 2014 (20 cm), Kiz-9 2015, Kiz-9 2014 (18.5 cm), Kiz-9 2013, Kiz-8 2015, Kiz-8 2014 (19.5 cm), Kiz-8 2013, Kiz-20 2013*). The experiment was performed with 2 solution of 1-propargyl-1-(2-methyl-3-oxo-3-(*p*-tolyl)propyl)-piperidin-1-ium bromide with different concentration – 0.01% (mass) and 0.001% (mass) and water was included as control. All dishes and experiment equipment were sterilized. Sorghum seeds were soaked in 90% ethanol for 6-7 minutes and after, were washed 3-4 times with distilled water. 20 seeds were placed in each Petri dish (10 Petri dishes for control, 50 – for each concentration of solution) on the filter paper, so they did not touch walls and each other. The filter paper was moisturized with the corresponding solution. The samples were placed in a light-proof cabinet, temperature: 22-25°C. Growth parameters including root length, shoot length and rate of the germination were determined after 4 and 9 days. For each batch of 100 seeds, the normally sprouted seeds are counted, considering the initial and final calculations. If the germination results of individual batches did not exceed the standard deviation, the batches were considered comparable. The result was the determination of the arithmetic mean with an accuracy of 1%.

3. Result and Discussion

Ultrasound activation - one of the best known non-conventional activation techniques at a laboratory scale. It is used by chemists to increase the reaction rate, speed up reaction time, and operate the reaction at more mild conditions [8]. Synthesis of 1-propargyl-1-(2-methyl-3-oxo-3-(*p*-tolyl)propyl)-piperidin-1-ium bromide (Figure 1) was carried out from obtained free-base [7] of tolperisone hydrochloride with *N*-alkylation under room temperature and with ultrasound activation (Table 1). The isolated yield of both methods is showed almost identical results; however, the reaction rate of the ultrasound activation method is 28 times faster.

During the assessment of the growth-stimulating activity of the synthesized ionic compound, the effect of the seed germination was studied on the 10 genotypes of sweet sorghum and growth parameters such as root length, shoot length, seed energy germination rate (after 4 days), and germination capacity (after 9 days) were determined. Experiments were carried out with solutions of 1-propargyl-1-(2-methyl-3-oxo-3-(*p*-tolyl)propyl)-piperidin-1-ium bromide (concentration 0.01% and 0.001%) and water were used as control. The mean value of the results on the effect of germination of sweet sorghum seeds, including standard deviation (SD) is presented in Tables 2-4.

The data of the gemmogenesis (Table 2) and the rhizogenesis (Table 3) presents that samples with low concentration (0.001% solution) is demonstrated the higher intensity for tested genotypes than control samples. 0.01% concentration of ionic compound shows better results (for root and shoot length) only for genotype *Victoria-4 20 cm 2014* after 9 days of germination (Figure 2) compared to control and lower concentration.

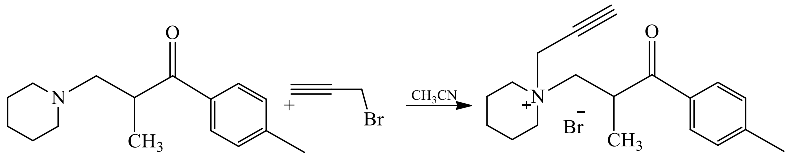

Figure 1 – The reaction of *N*-alkylation of tolperisone free-base with propargyl bromide to produce 1-propargyl-1-(2-methyl-3-oxo-3-(*p*-tolyl)propyl)-piperidin-1-ium bromide

Table 1 – The yield and time of the reaction in respect with method of synthesis

Method of synthesis	Time	Yield, %	Solvents of recrystallization	Melting point
Room temperature	6 days	81	Et_2O:EtOH = 1:4	180°C
Ultrasound activation	5 h	79	Et_2O:EtOH = 2:3	177°C

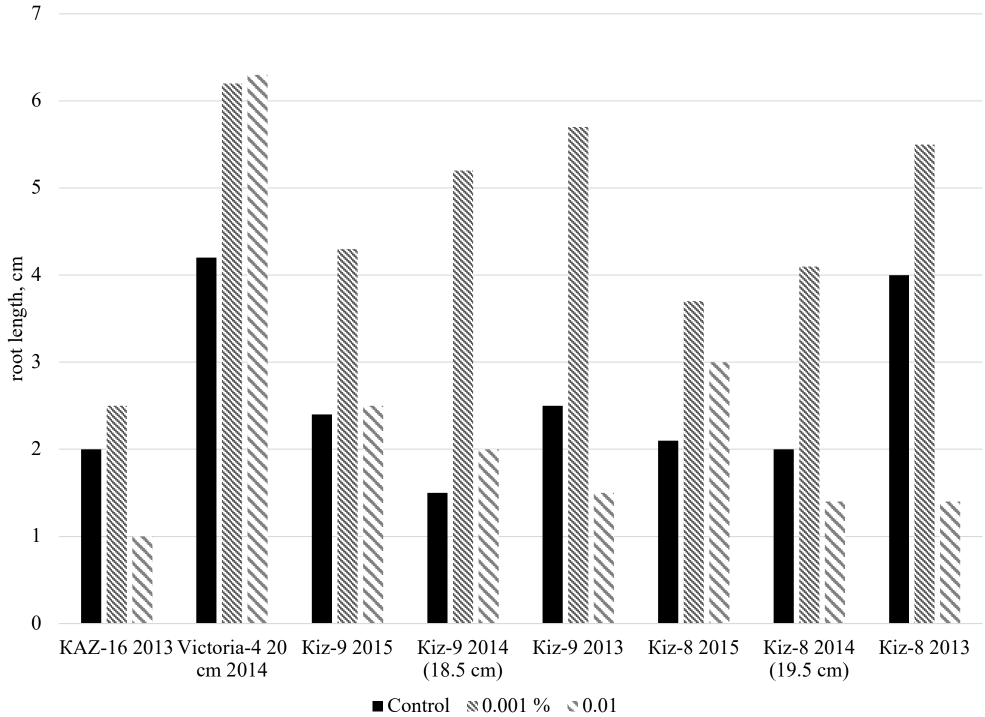

Figure 2 – Growth stimulant's effect on the root length (cm) of several genotypes of the sweet sorghum seeds after 9 days of germination

Table 2 – The result of 1-propargyl-1-(2-methyl-3-oxo-3-(*p*-tolyl)propyl)-piperidin-1-ium bromide's effect on the root length of sweet sorghum seeds

No.	Genotype	Germination after 4 days, cm (SD ±0.02)			Germination after 9 days, cm (SD ±0.3)		
		Control	0.01%	0.001%	Control	0.01%	0.001%
1	KAZ-16 2013	0.3	0.2	0.8	2.0	1.0	2.5
2	Victoria-4 2013	-	-	-	-	-	-
3	Victoria-4 20 cm 2014	1.2	1.5	1.8	4.2	6.3	6.2
4	Kiz-9 2015	0.1	0.2	0.1	2.4	2.5	4.3
5	Kiz-9 2014 (18.5 cm)	0.1	0.7	1.2	1.5	2.0	5.2
6	Kiz-9 2013	0.1	0.1	0.4	2.5	1.5	5.7
7	Kiz-8 2015	0.1	0.1	0.1	2.1	3.0	3.7
8	Kiz-8 2014 (19.5 cm)	-	0.1	0.1	2.0	1.4	4.1
9	Kiz-8 2013	0.1	0.1	0.1	4.0	1.4	5.5
10	Kiz-20 2013	-	-	-	-	-	-

Table 3 – The result of 1-propargyl-1-(2-methyl-3-oxo-3-(*p*-tolyl)propyl)-piperidin-1-ium bromide's effect on the shoot length of sweet sorghum seeds

No.	Genotype	Germination after 4 days, cm (SD ±0.01)			Germination after 9 days, cm (SD ±0.2)		
		Control	0.01%	0.001%	Control	0.01%	0.001%
1	KAZ-16 2013	0.5	0.1	1.0	2.5	2.0	4.5
2	Victoria-4 2013	-	-	-	-	-	-
3	Victoria-4 20 cm 2014	0.5	1.2	1.7	4.3	5.1	5.1
4	Kiz-9 2015	0.1	0.3	0.1	3.0	3.0	3.4
5	Kiz-9 2014 (18.5 cm)	0.1	0.4	0.5	3.0	3.5	4.5
6	Kiz-9 2013	0.1	0.2	0.3	3.3	2.0	4.7
7	Kiz-8 2015	0.1	0.1	0.1	3.2	4.2	5.0
8	Kiz-8 2014 (19.5 cm)	-	0.1	0.2	3.0	2.4	3.7
9	Kiz-8 2013	0.1	0.1	0.1	3.5	3.0	4.5
10	Kiz-20 2013	-	-	-	-	-	-

The result of seed energy germination rate (Table 4) varies depending on the genotype of sorghum. Samples with low concentration show a better result for *Kiz-9 2013, KAZ-16 2013, Kiz-8 2015* compared to control and higher concentrated solution. However higher concentrated solution is demonstrated by growth for 5-25% for such genotype as *Victoria-4 20 cm 2014, Kiz-9 2014 (18.5 cm)* and *Kiz-8 2014 (19.5 cm);* for genotype *Kiz-9 2015* and *Kiz-8 2013* water provides more effectiveness than solutions of ionic compounds. On the 9th day, we determined the germination capacity (Table 4), and it has been noticed that 4 genotypes (*KAZ-16 2013, Kiz-9 2014 (18.5 cm), Kiz-9 2013, Kiz-8 2015*) have maintained the same dependence of the result as seed energy germination rate. Nevertheless, *Kiz-9 2015, Kiz-8 2013, Victoria-4 20 cm 2014* presents the same rate of control and more concentrated solution of the ionic compound.

Table 4 – The result of 1-propargyl-1-(2-methyl-3-oxo-3-(*p*-tolyl)propyl)-piperidin-1-ium bromide's effect on the germination energy and germination capacity of sweet sorghum seeds

No.	Genotype	Germination energy, % (SD ±3.2)			Germination capacity, % (SD ±2.5)		
		Control	0.01%	0.001%	Control	0.01%	0.001%
1	KAZ-16 2013	60	35	80	65	40	80
2	Victoria-4 2013	-	-	-	-	-	-
3	Victoria-4 20 cm 2014	75	80	65	80	80	70
4	Kiz-9 2015	30	20	15	25	25	20
5	Kiz-9 2014 (18.5 cm)	30	50	25	20	55	30
6	Kiz-9 2013	30	30	55	30	35	55
7	Kiz-8 2015	15	15	35	50	50	70
8	Kiz-8 2014 (19.5 cm)	-	25	20	10	30	35
9	Kiz-8 2013	35	15	30	50	50	40
10	Kiz-20 2013	-	-	-	-	-	-

4. Conclusion

Using ultrasound activation for the synthesis of ionic compound, 1-propargyl-1-(2-methyl-3-oxo-3-(*p*-tolyl)propyl)-piperidin-1-ium bromide was obtained with almost the same yield in a shorter time. The synthesized ionic compound's solution with control did not show any effect on the germination of 2 genotypes: *Victoria-4 2013 and Kiz-20 2013*. For other genotypes of sweet sorghum (*KAZ-16 2013, Kiz-9 2014 (18.5 cm), Kiz-9 2013, Kiz-8 2015, Kiz-8 2014 (19.5 cm)*), germination energy and capacity of seeds, processes of gemmogenesis and intensity of rhizogenesis were better affected by solution with low concentration, although the influence of ionic compound on germination capacity of *Kiz-9 2015*, *Kiz-8 2013, Victoria-4 20 cm 2014* presents the same or worst results compared to control.

Acknowledgements

This Research has been funded by the Ministry of Education and Science of the Republic of Kazakhstan (Grants No. AP08857345 and AP08857516).

References (GOST)

1 Ali M.L., Rajewski J.F., Baenziger P.S., Gill K.S., Eskridge K.M., et al. Assessment of genetic diversity and relationship among a collection of US sweet sorghum germplasm BY ssr markers // Molecular Breeding. – 2007. – Vol.21, Is.4. – P.497-509.

2 Kleih U., Ravi S.B., Rao B.D., Yoganand B. Industrial Utilization of Sorghum in India // Monograph. International Crops Research Institute for the Semi-Arid Tropics. – 2000.

3 Somani R.B., Almodares A., Pandrangi R.B., Sepahi A. Potential of sweet sorghum in India and Iran for alcohol and sweetners production // First International Sweet Sorghum Conference, China. – 1997.

4 Ratnavathi C.V., Komala V.V., Lavanya U. Chapter 4 - Sorghum Uses—Ethanol. In: Ratnavathi C.V., Patil J.V., Chavan U.D. (Eds.) Sorghum Biochemistry. – Academic Press Elsevier, 2016. – P.181-252.

5 Ratnavathi C.V., Chavan U.D. Sorghum syrup and other by products // Sorghum Biochemistry. An Industrial Perspective. – 2016. – P.253-310.

6 Macedo W.R., Araújo D.K., Santos V.M., Castro P.R., Fernandes G.M. Plant growth regulators on sweet sorghum: Physiological and nutritional value analysis // Comunicata Scientiae. – 2017. – Vol.8, Is.1. – 170 p.

7 Giner B., Mergenbayeva S., Lomba L., Rafikova K., Dauletbakov A. et al. Synthesis and ecotoxicological studies of ionic compounds based on tolperisone, diphenhydramine and trimecaine // ChemistrySelect. – 2020. – Vol.5, Is.41. – P.12823-12828.

8 Lévêque J.M., Estager J., Draye M., Cravotto G., Boffa L., Bonrath W. Synthesis of ionic liquids using non conventional activation methods // An Overview. Monatshefte Für Chemie – Chemical Monthly. – 2007. – Vol.138, Is.11. – P.1103-1113.

References

1 Ali ML, Rajewski JF, Baenziger PS, Gill KS, Eskridge KM, et al (2007) Mol Breeding 21(4):497-509. *https://doi.org/10.1007/s11032-007-9149-z*

2 Kleih U, Ravi SB, Rao BD, Yoganand B (2000) Industrial Utilization of Sorghum in India. Monograph. International Crops Research Institute for the Semi-Arid Tropics.

3 Somani RB, Almodares A, Pandrangi RB, Sepahi A (1997) Potential of sweet sorghum in India and Iran for alcohol and sweetners production. First International Sweet Sorghum Conference, China.

4 Ratnavathi CV, Komala VV, Lavanya U (2016) Chapter 4 - Sorghum Uses—Ethanol. In: Ratnavathi CV, Patil JV, Chavan UD (Eds) Sorghum Biochemistry. An Industrial Perspective. Academic Press Elsevier, UK. P.181-252. *https://doi.org/10.1016/b978-0-12-803157-5.00004-6*

5 Ratnavathi CV, Chavan UD (2016) Sorghum Biochemistry 5:253-310. *https://doi.org/10.1016/b978-0-12-803157-5.00005-8*

6 Macedo WR, Araújo DK, Santos VM, Castro PR, Fernandes GM (2017) Comunicata Scientiae 8(1):170-175. *https://doi.org/10.14295/cs.v8i1.1315*

7 Giner B, Mergenbayeva S, Lomba L, Rafikova K, Dauletbakov A et al (2020) ChemistrySelect 41:12823-12828. *https://doi.org/10.1002/slct.202001771*

8 Lévêque JM, Estager J, Draye M, Cravotto G, Boffa L, Bonrath W (2007) Chemical Monthly 138(11):1103-1113. *https://doi.org/10.1007/s00706-007-0742-y*