UWAVE-2000 as an effective equipment for the microwave assistant synthesis of N, N-diethyl-2-(mesitylamino)-N-benzyl-oxoethanamonium chloride – a compound with plant growth regulat...

Article · October 2022

DOI: 10.55452/1998-6688-2022-19-3-45-53

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UDC 547-38
IRSTI 31.17
https://doi.org/10.55452/1998-6688-2022-19-3-45-53

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UWAVE-2000 AS AN EFFECTIVE EQUIPMENT FOR THE MICROWAVE ASSISTANT SYNTHESIS OF N,N-DIETHYL-2-(MESITYLAMINO)-N-BENZYL-OXOETHANAMONIUM CHLORIDE – A COMPOUND WITH PLANT GROWTH REGULATING ACTIVITY

Abstract. The synthesis of N,N-Diethyl-2-(mesitiylamino)-N-benzyl-oxoethanamonium chloride was carried out by N-alkylation with traditional method and using microwave and ultrasonic activation. The synthesized ionic compound is characterized by IR, NMR ^{1}H and ^{13}C methods, and its growth-stimulating activity has been tested on Zea Mays L. maize seeds. The synthesis of N,N-Diethyl-2-(mesitilamino)-N-benzyl-oxoethanamonium chloride using unconventional methods such as microwave and ultrasonic activation resulted in higher yields in a shorter period of time than traditional method. The highest yield (98%) was achieved by microwave activation of UWAVE-2000 in a very short time, while in the classical caseit was lower and the reaction time was twenty-four times longer. The synthesized ionic compound positively influenced the germination and growth energy of maize seeds. In samples treated with solution N,N-Diethyl-2-(mesitylamino)-N-benzyl- oxoethanaminium chloride of mould lesions was not so many, so, the treatment of maize seeds with trimecaine benzyl chloride not only stimulates the plant growth, but also prevent fungi mould formation.

Key words: trimecaine, ionic compound, microwave irradiation, ultrasound activation, maize, germinating capacity, germination energy, fungi mould.

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UWAVE-2000 ТИІМДІ МИКРОТОЛҚЫН ӘДІСІМЕН ӨСІМДІКТЕРДІҢ ӨСУІН РЕТТЕЙТІН БЕЛСЕНДІЛІГІ БАР ҚОСЫЛЫС -N, N-ДИЭТИЛ-2-(МЕЗИТИЛАМИНО)-N-БЕНЗИЛ-ОКСОЭТАНАМОНИЙ ХЛОРИДІН СИНТЕЗДЕУ

Аңдатпа. N,N-Диэтил-2-(мезитиламино)-N-бензил-оксоэтанамоний хлориді синтезі қалыпты жағдайда N-алкилдеу арқылы және микротолқынды сәулелену мен ультрадыбыстық жандандыруды қолдану нәтижесінде жүзеге асырылды. Синтезделген иондық қосылыс ИҚ ,^{1}H және ^{13}C ЯМР көмегімен сипатталды. Өсуі-стимуляторлық белсенділігі Zea Mays L. жүгері тұқымдарында сыналды. Микротолқынды және ультрадыбыстық жандандыру сияқты дәстүрлі емес әдістерді қолдану арқылы, N,N-Диэтил-2-(мезитиламино)-N-бензил-оксоэтанамоний хлорид синтезі, әдеттегі әдіске қарағанда қысқа мерзімде жоғары шығымда нәтиже көрсетті. Ең жоғары шығым, өте қысқа уақыт ішінде UWAVE-2000 микротолқынды күшейтіп қолдана отырып алынды. Ал классикалық жағдайда шығым төмен, реакция уақыты жиырма төрт есе көп болды. Синтезделген иондық қосылыс бақылаумен салыстырғанда жүгері тұқымының өну және өсу энергиясына жақсы әсер етті. N,N-диэтил-

2-(мезитиламино)-N-бензил-оксоэтанамоний хлоридінің ерітіндісімен өңделген үлгілерде зақымдану ошақтары аз болды. Сондықтан жүгері тұқымын тримекаин бензилхлоридімен өңдеу өсімдіктің өсуін ынталандырып қана қоймайды, сонымен қатар зең саңырауқұлақтарының пайда болуына жол бермейді.

Тірек сөздер: тримекаин, иондық қосылыс, жүгері, микротолқын, ультрадыбыс, өсу белсенділігі.

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UWAVE-2000 КАК ЭФФЕКТИВНОЕ ОБОРУДОВАНИЕ ДЛЯ ОСУЩЕСТВЛЕНИЯ СИНТЕЗА С ПОМОЩЬЮ МИКРОВОЛН N,N-ДИЭТИЛ-2-(МЕЗИТИЛАМИНО)-N-БЕНЗИЛ-ОКСОЭТАНАМОНИЯ ХЛОРИДА – СОЕДИНЕНИЯ С РОСТСТИМУЛИРУЮЩЕЙ АКТИВНОСТЬЮ

Аннотация. Синтез N,N-Диэтил-2-(мезитиламино)-N-бензил-оксоэтанамония хлорида проводили в обычных условиях путем N-алкилирования и с использованием микроволновой и ультразвуковой активации. Синтезированное ионное соединение охарактеризовано методами ИК, ЯМР ^{1}H и ^{13}C, его ростостимулирующая активность проверена на семенах кукурузы Zea Mays L. Синтез N,N-Диэтил-2-(мезитиламино)-N-бензил-оксоэтанамония хлорида с использованием нетрадиционных методов, таких как микроволновая и ультразвуковая активация, привел к более высоким выходам за более короткий период времени, чем при использовании традиционных методов. Самый высокий выход был получен при микроволновой активации (98%) UWAVE-2000 за очень короткое время, в то время как в классическом случае выход был ниже, а время реакции было в двадцать четыре раза больше. Синтезированное ионное соединение положительно влияло на всхожесть и энергию роста семян кукурузы по сравнению с контролем. В образцах, обработанных раствором N,N-Диэтил-2-(мезитиламино)-N-бензил-оксоэтанаминия хлорида, плесневых поражений было не так много, поэтому обработка семян кукурузы бензилхлоридом тримекаина не только стимулирует рост растений, но и предотвращает образование плесневых грибов.

Ключевые слова: тримекаин, ионное соединение, кукуруза, микроволны, ультразвук, ростстимулирующая активность.

Introduction

The traditional methods of synthesis that warms up reactions mix with conventional equipments, like a heating mantle, are not optimal for the yield of reaction, as a result of the hot surface of the reaction vessel, reagents, products and solvents decompose over time [1]. An efficient synthetic method that is getting more useful present days is microwaves (MW) irradiation [2]. The first publication about using microwave irradiation in organic synthesis was reported in 1986 by Smith et al [3]. Also, the use of ultrasound activation (US) can decrease reaction times, resulting in high yield, increased selectivity, and pure products in various applications of synthetic organic chemistry [4,5]. The US activation was used to accelerate many kinds of synthetic organic reactions [6,7].

Main provisions

Maize (Zea mays L.) is the third most important food crop globally after wheat and rice. In sub-Saharan Africa, tropical maize has traditionally been the main staple of the diet; 95 % of the maize grown is consumed directly as human food and as an important source of income for the resource-poor rural population [8].

Potassium, nitrogen, phosphorus fertilizers were previously used to stimulate maize growth [9]. It was found that ionic compounds of trimecaine also show growth stimulating activity, furthermore, these ionic compounds were found to be non-toxic, which make it promising sustainable and environmentally friendly growth stimulants. Herein we report about the synthesis and growth regulating activity of new ionic compound obtained via N-alkylation of trimecaine.

Methods and materials

The melting point of ionic compound was measured in an open capillary tube on an OptiMelt (Stanford Research System). The^{1}Hand ^{13}C NMR spectra were recorded using a NMReady 60 MHz spectrometer at 25 or 30^{o}C by using $CDCl_3$ as a solvents. IR spectrum was recorded on a spectrometer «Nicolet 5700 FT-IR» using KBr pellets. The progress of reaction and purity of product were checked using the TLC method on silica gel plates (SigmaAldrich®, Germany) with iodine vapors development. The diethylether : ethanol mixtures (4:1 V/V and 5:1V/V) were used as eluents. The TLC spots on the developed plates were observed in UV light (λ = 254 nm). All the reactants and solvents from SigmaAldrich®. An ultrasonic probe from Cole Parmer (50–60Hz, 0–240W) and a domestic U-WAVE-2000 generator (0–2000W) were used for the reaction. The separation and purification of substances was carried out by crystallization from appropriate solvents.

U-Wave 2000 have a three energy sources – microwave, ultrasonic, and ultraviolet irradiation can be free combined and work together, and software control timing on and off, achieving the synergistic effect by multi energies. UWave-2000 adopts immersion ultrasonic launcher, with an adjustable scope of ultrasound power: 0 – 800W, frequency of 28KHZ, and automatic frequency sweeping and frequency locking. With two sets of ultraviolet source (standard UV lamp power is 300W, dominant wavelength is 365nm, while optional UV lamp power is 100W, and dominant wavelength is 254nm), it can conduct selective photochemical research. UWave-2000 is equipped with multiple reaction vessels, 50-2000ml glass flasks can meet the routine use, and optional multi-standards quartz reaction flasks can meet photochemical reaction under the ultraviolet radiation.

N,N-Diethyl-2-(mesitylamino)-N-benzyl-oxoethanamine (trimecaine base) was synthesized from commercially available hydrochloride by neutralization. The equation for the synthesis of N,N-Diethyl-2-(mesitylamino)-N-benzyl-oxoethanamonium chloride from trimecaine base is given in Figure 1.

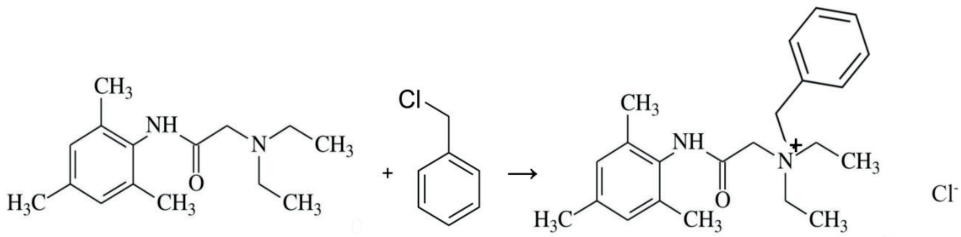

Figure 1 – Synthesis of N,N-Diethyl-2-(mesitylamino)-N-benzyl-oxoethanamonium chloride

Synthesis of the ionic compound was performed in the classical conditions and using microwave, ultrasound activation. The reaction time of trimecaine base with benzylchloride in conventional conditions (reflux in acetonitrile) and using ultrasound and microwaveactivation presented in Table 1 (Trimecaine:benzyl chloride mole ratiois 0.01:0.011). After the completion of the process, the volumeof the solution was halved by evaporation and cooled. The resulting isolated product was separated and purified by crystallization, and the purity of the product was checked by TLC using a mixtureof diethyl ether and ethanol (4:1) as eluent.

Synthesis, spectral and other data for theN,N-Diethyl-2-(mesitylamino)-N-benzyl- oxoethanamonium chloride.

Into the 100 ml flask 15 ml of acetonitrile was added and 0.01 mol of trimecaine base was dissolved. Thereafter the solution of 0.011 mol benzyl chloride was added and the resulted solution was kept under the reflux in conventional method (78–82^{o}C). The same mixture of solution were used in unconventional methods and the reaction mixture was placed in a US reactor and the contents reacted under US conditions characterized by the following parameters: US = 240 W at 30–40 °C, while the same type reaction mixture was placed in a UWAVE reactor and the contents reacted under MW conditions.

After recrystallization, the product is light yellow crystals. M.p. 171-173 °C. IR (KBr), cm^{-1} : 3173 (N-H), 1693 (C=O amide), 1528 ($C_{aromatic}$=$C_{aromatic}$). ^{13}C NMR ($CDCl_3$, 25^0C) δ, ppm: 162.51 (s, C=O); 136.75 (s, CH_3); 135.20 (s, CH_3); 131.27 (s, $C_{aromatic}$-NH); 129.02 (s, $C_{aromatic}$); 59.05 (s, CO-CH_2-N^+); 57.94 (s, N^+-CH_2-CH_3); 48.42 (s, N^+-CH_3); 21.03 (s, $C_{aromatic}$-CH_3); 18.55 (s, $C_{aromatic}$-CH_3); 8.37(s,N^+-CH_2-CH_3). ^1H NMR ($CDCl_3$, 25^0C) δ, ppm: 9.81(s, N-H); 6.87 (s, $H_{aromatic}$); 4.27 (s, CO-CH_2-N^+); 3.53, 3.54, 3.55 (N^+-CH_2-CH_3); 3.16 (s, N^+-CH_3); 2.19 (s, $C_{aromatic}$-CH_3); 2.08 (s, $C_{aromatic}$- CH_3); 1.30, 1.26, 1.24 (t, N^+-CH_2-CH_3). Calculated for $C_{19}H_{33}N_2OI$, %: C, 52.77; H, 7.64; N, 6.48; O, 3.70; I, 29.40. Found %: C, 51.81; H, 7.89

The synthesized compound was tested for germination energy and capacity with maize seeds. For this goal, 10^{-2} % (by mass) solution was prepared with the synthesized ionic compound. All dishes and other accessories were sterilized and kept in a laboratory oven 120 °C for 40 min. Maize seeds were sterilized with 90% ethanol for 6-8 min and washed three times with distilled water. For each assay, 10 Petri dishes were taken for control and 50 dishes (20 seeds per dish) for each compound solution. The seeds were planted immediately after storage at a given temperature. The seeds were placed so that they did not touch each other or the walls. The filter paper under the seeds was moistened with water for control and solutions of ionic compounds for testing. The samples prepared in this way were placed in a light-proof cabinet with a temperature of 22 to 25 °C. The germination energy and germinating capacity were evaluated in accordance with the standard. The germinated seeds were counted twice, that is, four and seven days after planting. 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%.

Results and discussion

In comparison with the traditional method (thermal activation), the using of microwave and ultrasound is a favorable method in organic synthesis, the acknowledgments has increased significantly in recent years in an attempt to understand the mechanism of action. Many applications in organic synthesis have made microwaves and ultrasound appealing for many researchers, and they are increasingly used in organic synthesis. These unconventional methods have turned out to be a good tool for achieving high yields and reducing reaction time. The results of the alkylation reaction conducted in this work under various reaction conditions confirmed the trends in the literature, and the results obtained collected and presented in table1.

Table 1 –The parameters of the reaction

Synthesis/reaction conditions	Time/min	Yield, %
Classic method	240	58%
US activation	120	56%
MW activation	10	98%

The highest isolated yield is reached under microwave irradiation in the shortest time, while in classical method the reaction time was twenty four times greater with a lower yield. Ultrasound activation showed approximately the same results, but the reaction rate was faster. To study the effect of synthesized ionic compound to germination energy and germinating capacity of maize seeds, experiments were conducted with 1 solution (concentration 10^{-2} %). The mean value of the results of 1 on the effect of germination energy and capacity of maize seeds with control (water) is presented in Table 2.

Table 2 – The results of germination energy and the degree of damage by mold fungi of maize seeds

(concentration of solutions are 10^{-2} %; root length and shoot length in cm)

Solutions	2 days	3 days	4 days	5 days	6 days	7 days	The degree of damage by mold fungi
Water	1	81	84	83	82	82	medium
Commercial Growth stimulator Akpinol		91	91	92	92	92	severe
N,N-Diethyl-2-(mesitylamino)-N-benzyl-oxoethanamonium chloride		91	**93**	93	92	91	**low**

According to the results of the experiment, based on the data on the energy of germination and germinating capacity, as well as the degree of damage by mold fungi, we assume that N,N-Diethyl-2-(mesitylamino)-N-benzyl-oxoethanamonium chloride exhibits growth-promoting activity comparable with the commercial plant growth regulator. The germinating capacity energy and germination of seeds treated with a solution of the substance N,N-Diethyl-2-(mesitylamino)-N-benzyl-oxoethanamonium chloride are 93%, and the degree of damage by mold fungi is fixed at the "low" level. An average of energy of germination and germinating capacity was found. This is shown below in Figure 2 for the investigated substances.

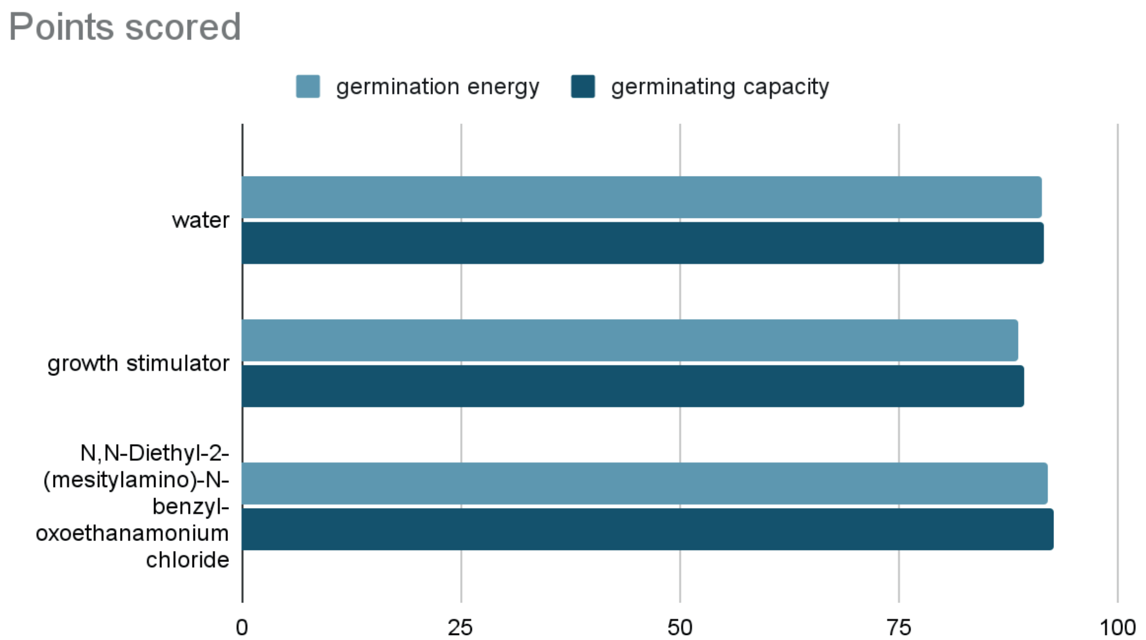

Figure 2 – An average of energy of germination and germinating capacity of water, commercial plant growth stimulator Akpinol and N,N-Diethyl-2-(mesitylamino)-N-benzyl-oxoethanamonium chloride for maize seeds

The seed sprouts of trimecaine benzyl chloride were significantly stronger and longer. In the first days of the experiment, the seeds treated with water grew very slowly. The samples treated with a solution of the growth stimulant Akpinol were drowned in mold. Figure 3 shows the average value for fungi mould damage.

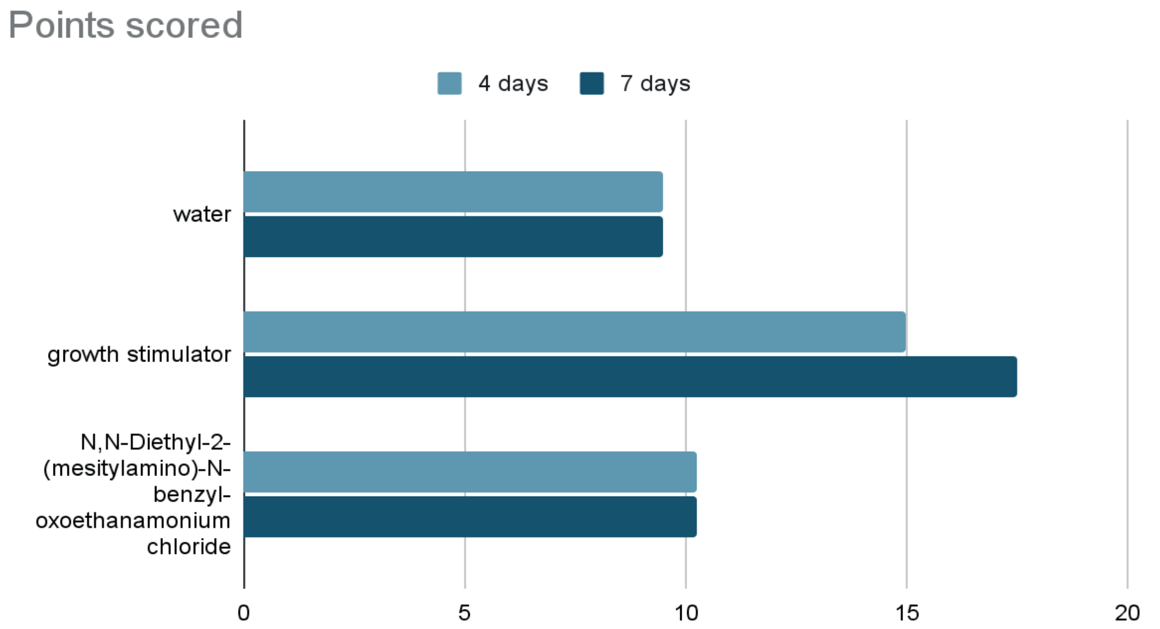

Figure 3 – Average value for fungi mould damage when maize seeds where treated with water, commercial plant growth stimulator Akpinol and N,N-Diethyl-2-(mesitylamino)-N-benzyl-oxoethanamonium chloride

During the observation, mouldy spots appeared on the filter paper, which later grew into a mould blanket that enveloped the entire cup. The growth stimulant Akpinol was distinguished by a high degree of damage to fungi mould. In samples treated with solution N,N-Diethyl-2-(mesitylamino)-N-benzyl-oxoethanamonium chloride of mould lesions was not so many, mostly seeds are characterised by blackening. So, the treatment of maize seeds with trimecaine benzyl chloride not only stimulates the plant growth, but also prevent fungi mould formation.

Conclusion

Using the UWAVE-2000 with microwave and ultrasound activation, ionic compoud N,N-Diethyl-2-(mesitylamino)-N-benzyl-oxoethanamonium chloride, trimecaine benzyl chloride, was isolated with a higher yield 98% (MW activation) in a more shorter time 60 min than the traditional method (thermal activation). The product with the highest yield is isolated under microwave irradiation, while in conventional method the yield was lower, 58%, and reaction time was twenty four times greater. The synthesized ionic compound better affected the germination energy and capacity for maize seeds compared with control. The germination energy in control (water) was 82% while in ionic compound studied it was 91%. The average value for fungi mould damage for control – 15-17,5 which is significantly larger than for the ionic compound studied – 10,25. So, the ionic compound N,N-Diethyl-2-(mesitylamino)-N-benzyl-oxoethanamonium chloride can be recommended for the further study as a potent plant growth stimulator which simultaneously prevents fungi mould growth.

Acknowledgements. This work was financially supported by the Ministry of Education and Science of Kazakhstan [AP08857516].

Conflict of Interest: The authors declare that they have no competing interests

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