PROCEEDINGS OF THE LATVIAN ACADEMY OF SCIENCES. Section B, Vol. 66 (2012), No. 1/2 (676/677), pp. 36–40.

DOI: 10.2478/v10046-011-0044-y

INFLUENCE OF WASTE LIQUORS OF MILDRONATE PRODUCTION ON YIELD AND QUALITY OF WINTER RAPESEED

Gunārs Bremanis and Solveiga Maļecka

State Stende Cereal Breeding Institute, "Dižzemes", Dižstende, Lībagu pag., Talsu nov., LV-3258, LATVIA
stende.selekcija@apollo.lv

Communicated by Ivars Kalviņš

*The effect of mother liquors obtained in one production stage of the popular medicinal preparation Mildronate on yield and quality of winter rape (*Brassica napus *L.) grown for biofuel was tested. The mother liquors contain quaterine-type compounds, which are known to stimulate growth of lupines. In the three-year study, Mildronate mother liquors were sprayed on winter rape. The yield and fat content of rape was determined. Mother liquor contains small amounts of methyl acrylate. However, this compound was not found in rapeseed, stalks and soil. Mother liquors spray had a positive effect on soil microorganisms. In the period from initiation of flowering to maximum flowering stage, spraying with mother liquors, as well as with quaterine-M and isopropanol (basic substance of mother liquor), in most cases had a low (around 10%) positive effect on rape yield and a very small impact on the fat content in rape grain. The effects differed among years, suggesting interaction with weather conditions. The determined optimal mother liquors dosages were quite wide, from 0.4 to 10 L ha^{-1}.*

Key words: *rape, mildronate, spraying, Grindeks.*

INTRODUCTION

The adaptogenous activity of the medicinal preparation "quaterine", produced in the Latvian Institute of Organic Synthesis, on plants and particularly on lupines is already known (Shutov *et al*., 1980; Trapencier *et al*., 1981). During the vegetation period of field crops, conditions can occur that are unfavourable to their growth and development: weather conditions, invasion of pests, competition from weeds; sometimes excessive or inappropriate usage of pesticides. Due to their adaptogenous traits, quaterine, its derivatives and production by-products containing quaterine-type compounds may be able to promote plant growth in unfavourable growth periods, as previously observed on lupine yield (Shutov *et al*., 1980). Production of Mildronate at the JSC Grindeks results in potentially valuable derivatives of quaterine, which currently are utilised.

We studied the effect of mother liquors, obtained in one production stage of mildronate, on rape growth. Since the mother liquor is basically isopropanol, and contains small amounts of methyl iodide and methyl acrylate, we chose to use a technical culture: winter and spring rape. The area this crop planted has much increased, due to its potential application for biofuel.

A review of literature did not register any examples of application of an adaptogen in agriculture or rapeseed sowing, but studies are being conducted on adaptogens of natural origin and their use in medicine. Growth stimulators of natural origin which contain active, well-known substances, such as cytokinins or auxins have been applied in agriculture agricultural use (Budzyński, 2008; Krawczyk and Skoczyński, 2008; Malarz; 2008; Matysiak and Sylwia Kaczmarek, 2008) due to their specific impact, rather than adaptogenous effect. Fungi belonging to *Fusarium* contain the toxin Zearalenon, which has oestrogenic properties and has effect similar to auxin (Biesaga-Koscielniak and Filek, 2010).

The aim of the current research was to test application of a manufacturing by-product, which currently causes losses due to its utilisation costs, on yield and quality of rape.

MATERIALS AND METHODS

Winter rape was cultivated in field conditions at the State Stende Cereals Breeding Institute from 2007 to 2009. In the season of 2006/2007, the rape was grown on sandy loam soil with a podzolic turf (pH 5.1–5.5); in 2007/2008, on sandy loam soil with a podzolic turf (pH 5.9–6.9); and in 2008/2009, on sandy loam soil with podzolic turf and turf gley (pH 6.2–6.7). The varieties used were 'Celsioss' in the first season and 'Banyo' in the second and third season.

Winter rape trials were carried out in prepared fields for sowing, choosing smooth places with homogenous soil characteristics. The doses of mother liquors of mildronate used were: 40 ml (during the first season), 80 ml (during the second and the third season), and also 400 ml, 2000 ml and 10 000 ml per hectare. The respective doses of quaterine were: 2 g (during the first season), 4 g (during the second and the third season), and 20 g, 100 g and 500 g per hectare. Spraying was performed in different rape development stages. In the first season the first spraying was conducted during the middle of the flowering period (61–63 ZS), the second spraying — at the very end of flowering in the pod development stage (69–70 ZS). An additional variant was spraying in both above times. The spraying was performed using a trial sprayer and applied with compressed water. The solution was prepared by dissolving the preparation dose 2 l water. The winter rape was harvested on 31 July 2007, 31 July 2008, and 10 August 2009 (Table 1).

Plot yield, 1000 kernel weight and fat content were measured in all seasons.

Significant differences were tested using analysis of variance (ANOVA: two-way with replications, ANOVA: two-way without replications, and ANOVA: one-way).

Taking into consideration the results of the first year, the dose in the second research year was increased to 80 ml, accordingly, 4 g per hectare, and the spraying period was extended, by performing the first spraying at the end of footstalk rising stage, which occurred at the beginning of budding stage.

During the third year, taking into account the previous results, spraying was conducted at two times with the same doses as in the previous year, but a new variant was used for comparison: the solution was supplemented with pure isopropanol. Thus, the effects of mother liquors, quaterine-M, pure isopropanol were compared to a control.

To test environmental effect of mother liquors of mildronate, the presence of methyl acrylate in soil, rape grain, oil and footstalk was tested, as well as changes of soil microorganisms in sprayed rape plots. Also, regular monitoring of sprayed rape and rape leaf scanning was conducted.

Meteorological conditions. Meteorological data were obtained from the Stende Hydro Meteorological Station. Autumn of 2006/2007 was favourable for development of rape, but the beginning of winter was atypically warm with abundant precipitation. Also the first and the second part of Jan-

Table 1

METHODS AND CONDITIONS OF WINTER RAPE TRIALS

Agrotechnical element	2007	2008	2009
Variety	'Celsioss'	'Banyo'	'Banyo'
Soil characteristic	sandy loam soil with podzolic turf	sandy loam soil with podzolic turf	sandy loam soil with podzolic turf
$pH_{(KCl)}$	5.1–5.5	5.9–6.0	6.2–6.7
Content of organic matter, g kg^{-1}	19–26	22–30	22–30
AvailableP_2O_5, mg kg^{-1} (DL method)	92–124	209–280	89–393
AvailableK_2O, mg kg^{-1} (DL method)	98–100	131–158	79–168
Precrop	buckwheat for manure	buckwheat for manure	buckwheat for manure
Fertiliser, N-P-K	10-20-20, 230 kg ha^{-1}	4-20-20, 300 kg ha^{-1}	6-26-30, 300 kg ha^{-1}
Sowing rate and time	4.0 kg ha^{-1}, 16.08.2006	3.5 kg ha^{-1}, 16.08.2007	2.7 kg ha^{-1}, 19.08.2008
Fertiliser	NH_4NO_3 480 kg ha^{-1} in two times (renewal of plant vegetation and footstalks' formation)	NH_4NO_3 426 kg ha^{-1} in two times (renewal of plant vegetation and footstalks' formation)	NH_4NO_3 420 kg ha^{-1} in two times (renewal of plant vegetation and footstalks' formation)
Herbicide	Active ingredient metazahlor (Butisan Star) 2.5 L ha^{-1}, 17.08.2006	Active ingredient metazahlor (Butisan Star) 2.5 L ha^{-1}, 19.08.2007	Active ingredient metazahlor (Butisan-400) 2.5 L ha^{-1}, 19.08.2008
Retardant	Active ingredient tebukonazol (Folicur) 1.0 L ha^{-1}, 18.09.2006	Active ingredient tebukonazol (Folicur) 0.8 L ha^{-1}, 7.05.2008	Active ingredient tebukonazol (Folicur) 0.6 L ha^{-1}, 10.10.2008
Insecticide	Active ingredient alfa-cipermetrin (Fastac 50) 250 ml ha^{-1}, 27.04.2007	Active ingredients tiakloprin & deltamethrin (Proteus 0.6) L ha^{-1} 24.04.2008 & 0.6l ha^{-1} 7.05.2008	Active ingredient alfa-cipermetrin (Fastac 50) 250 ml ha^{-1}, 29.04.2009
Variant	24 and 6 control plots, 4 replications	32 and 10 control plots, 5 replications	32 and 10 control plots, 5 replications
Number of plots	102, size of plots 6 m^2	170, size of plots 15 m^2	170, size of plots 15 m^2

uary were atypically warm. At the end of winter, temperature decreased; the snow layer was comparatively thin, causing damage to the crop in places where there was no snow. The lowest air temperature (–19.7 °C) was recorded in the end of January, when the soil was covered by a 16-cm layer of snow. At the beginning of February, temperature became milder, and snow rapidly melted. Temperature in the second part of February decreased and remained cold all month. In general, the wintering conditions were unfavourable. The third part of March was very warm and the vegetation rejuvenated in winter crops, but the beginning of April was comparatively colder than March with frosts during nights. Many plants were damaged during the winter. Growth of winter crops completely revived only in the third part of March. Conditions for sowing of spring rape were favourable. The beginning of March was cool and dry; nights were frosty. The weather at the end of May was very warm. In general, weather for growth and development of rape was satisfactory. Due to the warm weather the stem elongation phase occurred rapidly. June was warm and dry. Average air temperature in July was close to the norm; August also was warm, but mostly rainy. Conditions for winter rape sowing then were optimal. At the beginning of winter 2007/2008, the weather was atypically warm. January and February were atypically warm for winter and wintering conditions for the crops were satisfactory. March was warm and wet; the average temperature was 2.5°C higher than the norm. April started with warm weather and winter crops revived, but temperature fell in the beginning in the second part of a month and vegetation in winter crops stopped growth for a period. May was comparatively mild and dry, nights were frosty, but the end of May was very warm. Weather for growth and development of rape was satisfactory. Due to warm weather rape rapidly grew and started to flower. The weather remained warm and dry also in June, resulting in less available. The average air temperature in July was close to the norm. August was warm, but rainy. Harvest was possible only in rainless days. Generally, precipitation sum in August was 159.5 mm, which was 183.3% compared to the norm. The conditions for germination and development were favourable.

Autumn of 2008/2009 was comparatively warm, and the greatest precipitation sum was observed in October. Air temperature in November was above the norm, and precipitation was 172% higher than the. Also the average air temperature in December was above the norm. The average air temperature in January was –4.6 °C. The snow layer was not firm and wintering conditions was satisfactory. Weather in February was cool, precipitation was in form of snow or wet snow, and wintering conditions were favourable. March was cool, but the average temperature was higher than norm. The beginning of April was warm and dry and such weather lasted all month; although nights were frosty. May was comparatively warm with a small precipitation amount. The beginning of June was warm and humid; moisture was sufficient. In general, the temperature in June was lower than the norm. July was warm and sufficiently humid. The weather at the beginning of August was warm and dry, the middle of this month was rainy, and the end — sunny.

RESULTS

The impact of mother liquors of mildronate on rape yield and fat content differed between the three years of study, depended on agro-climatic conditions. During first season, the largest doses of mother liquors of mildronate (2 l and 10 l per ha) had a significant positive effect on yield of winter rape, in cases when spraying was performed in the middle of flowering period and when two sprying times were implemented — in the middle and at the very end of flowering (in both variants the dose was 400 ml per ha). Spraying at the very end of the flowering period, as well as spraying with a lower dose like 80 ml per ha, did not have significant effect on yield. Analysis of variance (probability <95%) did not show and effect of dose, but spraying time had significant effect ($F = 5.76 > F_{0.01} = 5.31$). Dose and time of spraying Quaterine-M did not have significant effect on rape yield (Fig. 1).

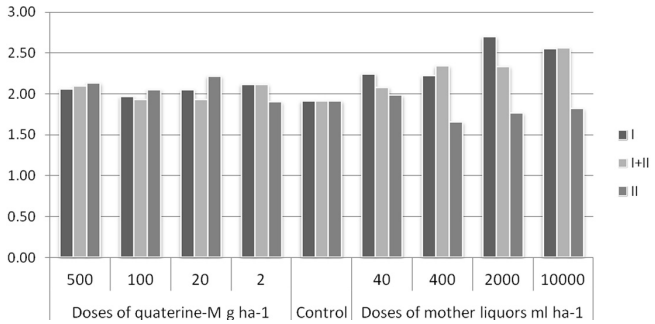

Fig. 1. Yield of winter rape in 2007 (t ha^{-1}). Spraying: I – the first, II – the second, I + II, both.

In 2008, no significant effect of dose and time of spraying with mother liquors of mildronate and of quaterine-M on yield of winter rape was observed, when compared to the control control (mother liquors: $F = 2.36 < F_{0.05} = 2.49$; quaterine-M: $F = 1.69 < F_{0.05} = 2.49$).

In 2009, only optimal spraying times were implemented and the effect of pure isopropanol was tested.

The effect of dose ($F = 3.66 > F_{0.01} = 3.49$) on winter rape yield in 2009 was significant. The effect of spraying time ($F = 4.56 > F_{0.05} = 3.92$) did not reach the level of significance, but there was a tendency that in most cases rape yield was bigger in variants with the first spraying (first flowers, beginning of flowering stage); only in case of isopropanol did the late spraying (in flowering maximum) lead to improved effect. The most effective doses of the tested chemical agents were second and third doses. The first sprayings of isopropanol with the second (0.4 L ha^{-1}) and third (2 L ha^{-1}) doses provided significantly better yield than of the control. In the case of quaterine-M, the second dose (20 g ha^{-1}) was most effective for both first and second spraying. Spraying of mother liquors significantly improved

winter rape yield both utilising the first and second spraying with the second (0.4 L ha^{-1}) and third (2 L ha^{-1}) doses. Spraying with mother liquors resulted in significantly better effect than with quaterine-M in both sprayings using the third dose and better than isopropanol in the second spraying of second dose. However, in general differences among sprayings of quaterine-M, isopropanol and mother liquors of mildronate were not significant (Fig. 2).

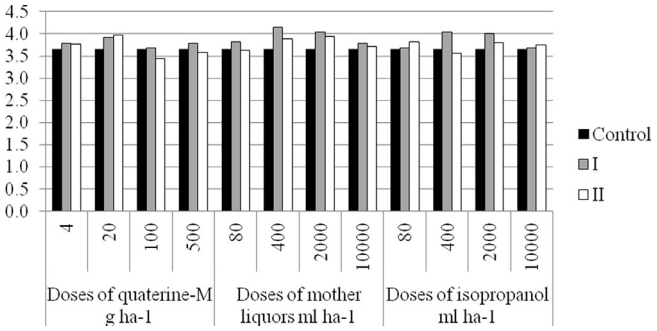

Fig. 2. Yield of winter rape in 2009 (t ha^{-1}). Sprayings: I – the first, II – the second.

Also the impact of spraying on fat content in rape depended on weather conditions in the corresponding season. In the season of 2007 the fat content in rape was higher than in the control in all variants, except in one. However, a significant difference was observed in only half of trials when sprayed with mother liquors, and only in one sixth of trials when sprayed with quaterine-M. Also, there was no significant difference for spraying time (mother liquor: $F = 0.105 < F_{0.05} = 4.459$; quaterine-M: $F = 0.619 < F_{0.05} = 4.459$). Also, the significance of dose reached only 91.4% for mother liquors and 85.8% for spraying with quaterine-M (respectively: $F = 3.021 < F_{0.05} = 3.838$ and $F = 2.347 < F_{0.05} = 3.838$).

In contrast to the previous season, in 2008, spraying doses of mother liquor and quaterine-M showed significant impact on fat content in rape grain (mother liquor: $F = 5.05 > F_{0.05} = 3.26$; quaterine-M: $F = 5.13 > F_{0.05} = 3.26$): the second dose of quaterine-M and second dose of mother liquor in all spraying times caused significantly reduced fat content.

In general, spraying time did not significantly affect fat content in rape grain: mother liquor: $F = 1.22 < F_{0.05} = 3.49$; quaterine-M: $F = 0.63 < F_{0.05} = 3.49$. The last spraying, when rape was sprayed with pure isopropanol, led to higher fat content in rape grain compared with the effect of mother liquors. This spraying was conducted without replications, and the effect will be studied in subsequent trials.

In 2009, the spraying dose showed significant effect on winter rape ($F = 12.03 > F_{0.01} = 3.49$). The choice of agent and spraying time did not have significant effect on fat content: the second dose (20 g ha^{-1}) and the third dose (100 g ha^{-1}) of quaterine-M in both sprayings caused better effect than the control, as did all sprayings with isopropanol and all sprayings with mother liquors, excluding the first spraying of first dose (80 ml ha^{-1}) and second spraying of the fourth dose (10 l ha^{-1}) (Fig. 3). The yield of rape fat (oil) per hectare was also calculated (Fig. 4).

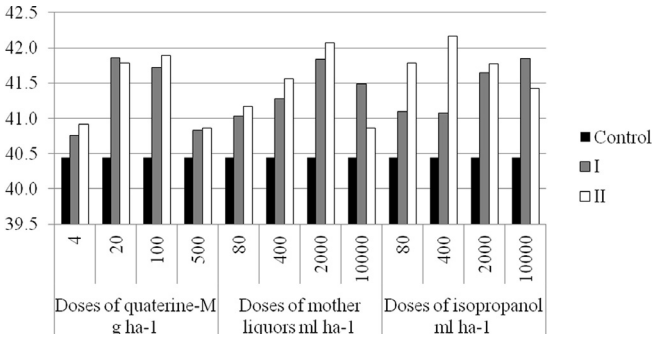

Fig. 3. Fat content in winter rape in 2009 (%). Spraying: I – the first, II – the second.

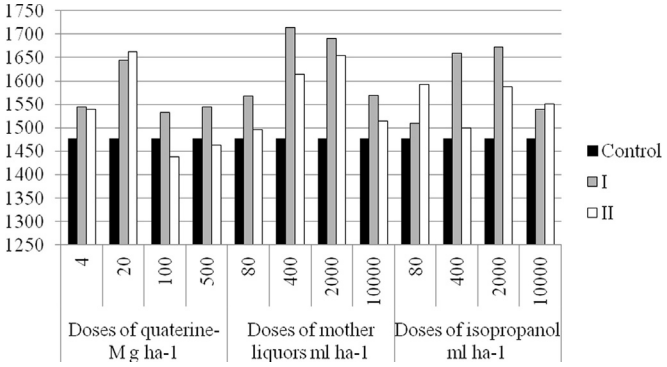

Fig. 4. Fat (oil) yield in winter rape in 2009 (kg ha^{-1}). Spraying: I – the first, II – the second.

In 2009, production trials with winter rape were performed, in which four variants were compared: spraying with mother liquor (10 l ha^{-1}, 0.5856 ha), with pure isopropanol (10 l ha^{-1}, 0.5856 ha), with quaterine-M (500 g ha^{-1}, 0.5760 ha), and control — sowing without spraying. The spraying was performed together with the first spraying in the experimental field, i.e. at the very beginning of the flowering stage. Control — in two fields with a total area of 0.9 ha (0.4712 ha + 0.4464 ha). The yield from the field sprayed with isopropanol exceeded that of the control by 3%, with quaterine-M by 5%, but with mother liquor of mildronate by 73% (Table 2).

Table 2

YIELD OF WINTER RAPE IN PRODUCTION TRIAL OF 2009

Variant	Area, ha	Yield, t at 9% humidity	Yield, t ha^{-1} at 9% humidity	Dried sample not purified g	Dried cleaned Sample, g	Yield of cleaned, t ha^{-1} (9% humidity)	%
Control	0.9176	2.02	4.29	1531.3	1385.2	3.81	100
Mother liquor	0.5856	4.40	7.51	1078.6	949.0	6.60	173
Quaterine-M	0.5760	2.55	4.43	843.1	765.3	4.02	105
Isopropanol	0.5856	2.64	4.50	1243.5	1080.8	3.91	103

DISCUSSION

Spraying with mother liquor of mildronate increased winter rape yield, except in some variants. The increase in most cases was around 10%, and depended on meteorological conditions in the season. Similar results were obtained forpure isopropanol and quaterin-M. Since the production trial was performed without replication, it is likely that the positive result for mother liquor of mildronate is a chance effect, which was affected by heterogeneity of fields in association with weather conditions during winter and the vegetation period. The production trial should be repeated, most likely in homogenous fields of Zemgale.

Spraying with mother liquor increased the fat content in rape seed, except in some variants, but mostly the observed difference from the control was insignificant, and sometimes a small decrease was observed. Obviously, the effect is very much affected by meteorological conditions.

The most optimal dose was 2 l ha^{-1}, but spraying can be conducted using a comparatively wide amplitude of doses — from 0.4 l ha^{-1} to 10 l ha^{-1}. The most optimal spraying time was from the appearance of first buds to the middle of flowering.

Methyl acrylate was not found in rape seed, footstalks, and soil in fields sprayed with mother liquor of Mildronate. The sprayings both with mother liquors and quaterine-M were visually evaluated to not affect bee behaviour and there are no visual physiological changes in plants. Analysis of soil microorganisms, performed in the Laboratory of Microbiology of Latvia University of Agriculture, found a slightly positive but temporary effect of mother liquor of Mildronate on soil.

There were no problems in compatibility of working liquids obtained from quaterine-M and mother liquors with 17 different frequently used pesticides. Mother liquor in doses from 0.4 to 10 l ha^{-1} are recommended for winter rape that is used biogas production. The spraying can be performed from the end of bud formation stage till the maximum of flowering, taking into consideration conditions for work with pesticides. Due to the bad smell of the mother liquor, it is necessary to avoid spraying in close to towns.

ACKNOWLEDGMENTS

The research was supported by grants from the Latvian Government and financial support from JSC "Grindeks". (TOP project Nr. 07-06).

REFERENCES

Biesaga-Kościelniak, J., Filek, M. (2010). Occurrence and Physiology of Zearalenone as a New Plant Hormone. *Soc. Org. Farm. Climate Change Soil Sci.: Sust. Agric. Rev.,* **3**, 419–435.

Budzyński, W., Dubis, B., Jankowski, K. (2008). Response of winter oilseed rape to the biostimulator Asahi SL aplied in spring. In: *Biostimulators in Modern Agriculture; Field Crops* (pp. 18–24). Dabrowski, Z.T. (Ed.). Warshaw: Wieš Jutra.

Krawczyk, R., Skoczyński, J. (2008). Winter survival and yield of oilseed rape depending on sowing date and application of micronutrient preparation route acting as a growth stimulator. In: *Biostimulators in Modern Agriculture; Field Crops* (pp. 33–40). Dabrowski, Z.T. (Ed.). Warshaw: Wiea' Jutra.

Malarz, W., Kozak, M., Kotecki, A. (2008). The use of Asahi SL biostimulator in spring rape growing. In: *Biostimulators in Modern Agriculture; Field Crops* (pp. 25–32). Dabrowski, Z.T. (Ed.). Warshaw: Wies' Jutra.

Matysiak, K., Kaczmarek, S. (2008). Potential advantages of Kelpak bioregulator applied to some field crops. In: *Biostimulators in Modern Agriculture; Field Crops* (pp. 99–106). Dabrowski, Z.T. (Ed.). Warshaw: Wieś Jutra.

Трапенциер П.Т., Калвиньш И.Я., Шутов Г.К., Бременис Г.А., Приеде Х.К., Мелберг Я.В., Каусс В.Я., Дирненс В. В. (1981). Соли 3-(2,2,2-триметил гидразиний) пропионовой кислоты, обладающие росторегулирующей активностью в отношении люпина [Salts of 3-(2,2,2-trimethylhydrazinium) propionic acid with growth's regulating activity to lupine]. A. c. 849724 СССР, 23.03.81 (in Russian).

Шутов Г.К., Калвиньш И.Я., Трапенциер П.Т. (1980). Способ регулирования роста растений люпина [Growth's regulation mode for plants of lupine]. A. c. 978808 СССР, 18.01.80., Б. и. № 45 (in Russian).

Received 17 November 2011

MILDRONĀTA RAŽOŠANAS ATSĀĻŅA IETEKME UZ ZIEMAS RAPŠA RAŽU UN KVALITĀTI

Veikts pētījums par populārā preparāta mildronāta vienas ražošanas stadijas atsāļņa pārbaudi uz biodegvielas ražošanai audzētu ziemas rapsi. Pētījumu pamato kvaterīna tipa savienojumu klātbūtne pētītajā atsālnī un fakts, ka kvaterīnam piemīt adaptogēnas īpašības un ka tas veicina lupīnas augšanu. Mūsu pētījumā trīs gadu laikā pārbaudījām mildronāta atsāļņa miglojumu ietekmi uz ziemas rapša ražu un tauku saturu rapša sēklās. Tā kā atsālnis satur nedaudz metilakrilāta piemaisījuma, pasūtījām pārbaudi uz metilakrilāta klātbūtni rapša sēklās, stublājos un augsnē, un to nekonstatējām. Pārbaudē tika konstatēta atsāļņa miglojuma ietekme uz augsnes mikroorganismiem, kas tika vērtēta kā visumā pozitīva. Noskaidrojām, ka laikā no rapša ziedēšanas paša sākuma līdz ziedēšanas fāzes maksimumam, kad pumpuru vēl ir vairāk nekā aizmetušos pākšu, miglojumi ar atsālni, kā arī salīdzināšanai ņemtie miglojumi ar kvaterīnu-M un izopropanolu — atsāļņa pamatvielu — vairumā gadījumu dod nelielu, līdz 10% pozitīvu efektu uz rapša ražu un pavisam nelielu — uz rapša graudu tauku saturu, taču iegūtie rezultāti bija atšķirīgi pa gadiem. Tas liecina, ka sevišķi liela loma ir metereoloģisko apstākļu ietekmei. Optimālās atsāļņa devas robežas ir visai plašas, no 0.4 līdz 10 L ha^{-1}.