NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 38, 2021
Print ISSN 1222–4227; Online ISSN 2067–5720

EFFECT OF FOLIAR ANTIBROADLEAF HERBICIDES ON FAT CONTENT IN SEEDS OF VARIOUS COTTON (*Gossypium hirsutum* L.) CULTIVARS

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ABSTRACT

In this study was investigate the effect of vegetative treatment with the herbicides Basagran 480 SL, Pulsar 40 and Express 50 WG on the fat content of seeds of different Bulgarian cotton cultivars. The experiment was carried out with twelve Bulgarian cotton cultivars (*Gossypium hirsutum* L.) - Chirpan-539, Helius, Trakia, Vicky, Philipopolis, IPK-Veno, Boyana, Avangard, Natalia, Darmy, Dorina and Nelina. The herbicides were applied at the bud formation stage of cotton. Fat content in cotton seeds was determined by extraction - SR ISO 6492 method. For the first time in the world it has been established that in the vegetative treatment with herbicides the most valuable with regard to cotton seed fat content are all cultivars treated with Basagran 480 SL, except Chirpan-539 and Helius. It has been established that the treatment with the Express 50 WG herbicide less affected the cultivars Chirpan-539, Helius, Trakia, Vicky, Philipopolis, IPK-Veno, Boyana, Avangard, Natalia but all cultivars were negative influenced by the treatment with the herbicide Pulsar 40 with regard to fat content in seeds.

Keywords: cotton, vegetation antibroadleaf herbicides, cultivars, fat content.

INTRODUCTION

Cotton is a crop characterized by long vegetation period and a poor competitive ability to weeds. Because of this, it is highly sensitive to weed spread from the earliest stages of its development.

Problems with primary weed spread in cotton are solved to a considerable extent (Chachalis and Galanis, 2007; Cardoso, 2011). The issue of secondary weed spread of annual and perennial graminaceous weeds during cotton vegetation is also solved to a great extent by using antigraminaceous herbicides (Gao, 2005).

Data on herbicides for efficient control of secondary emerging annual and perennial broadleaf weeds in conventional cotton growing technology are rather scarce even on a global scale. Effective herbicides for their control in cotton are still being sought. In the application of vegetative antibroadleaf herbicides in conventional technology, there are often manifestations of phytotoxicity (Barakova and Delchev, 2016; Barakova, 2017).

Information on glyphosate-tolerant cotton cultivars is presented (Gaylon et al., 2015; Spielman et al., 2015). In them control of all weeds - graminaceous and broadleaf, annual and perennial is completely solved by the use of total herbicides based on glyphosate (Roundup Ready technology) or glufosinate (Liberty Link technology). These technologies are widely used in major cotton-producing countries. However, these cultivars are GMOs and are banned within the territory of the European Union, incl. Bulgaria. This makes the present study particularly relevant not only for Bulgaria, but also for all cotton producing countries within the European Union.

The seeds of cotton are quite rich in fat - 17-23%. Cotton oil is one of the most important semi-dry oils in the world used for alimentary and technical purposes.

Received 21 February 2020; accepted 3 November 2020. First Online: November, 2020. DII 2067-5720 RAR 2021-8

Worldwide, there are many studies on the fat content in cotton seed, its quality and its purpose (Ataullaev et al., 1982; Hakoomat, 2005; Ashok, 2006; Constantin, 2007; Saldzhieva et al., 2008; Saldzhieva et al., 2009; Uzunova, 2008). Scientific literature does not yet know whether treatment with herbicides during cotton growing affects the fat content of cotton seeds.

The purpose of this study is to investigate the effect of vegetative treatment with the herbicides Basagran 480 SL, Pulsar 40 and Express 50 WG on the fat content of seeds of different Bulgarian cotton (*Gossypium hirsutum* L.) cultivars.

MATERIAL AND METHODS

During the period 2013-2015 a field experiment was conducted in non-irrigated conditions, on pellic vertisol soil type. The experiment was carried out with twelve Bulgarian cotton cultivars - Chirpan-539, Helius, Trakia, Vicky, Philipopolis, IPK-Veno, Boyana, Avangard, Natalia, Darmy, Dorina and Nelina belonging to *Gossypium hirsutum* L. species. These cultivars are not genetically modified. They were created by interspecies hybridization and experimental mutagenesis.

Herbicides Basagran 480 SL (bentazone), Pulsar 40 (imazamox) and Express 50 SX (tribenuron-methyl) were studied. They were applied at the bud formation stage of cotton. Spraying was made with a hand back sprayer with a work solution of 300 l/ha. The herbicides were applied against the background of the herbicidal combination Dual gold 960 EC (S-metolachlor) + Goal 2 E (oxyfluorfen), applied after sowing pre-emergence to control primary weed infestation of cotton. The experiment variants are given in Table 1.

In this study, calculation of adjusted stability variance ($Sh\text{-}S_i^2$) was necessary, because the heterogeneity term was significant (p<0.01). The stability statistic $Sh\text{-}Si^2$ calculated following removal of heterogeneity due to environmental index ($Z_j = \bar{X}_{.j} - \bar{X}_{..}$) as a covariate from GE interaction variance, where $\bar{X}_{.j}$ = mean of all cultivars in j^{th} environment of all cultivars across all environments, using the following equation (Shukla, 1972):

$$Sh - S_i^2 = \left[{}^{t}/(t-2)(e-2)\right] \times \left[s_i - \sum_{i=1}^{t} {}^{s_i}/t(t-1)\right]$$

where:

$$s_i = \sum_{j=1}^{s} (u_{ij} - \bar{u}_{i.} - b_i Z_j)^2$$

$$b_i = \sum_{j=1}^{s}[(u_{ij} - u_{i.})Z_j] \Big/ \sum_{j=1}^{s} Z_j^2$$

Cultivar stability across multiple years and locations was also evaluated using the ecovalence (W_i) (Wricke, 1962):

$$W_i^2 = \sum_{i=1}^{t} (X_{ij} - \bar{X}_{i.} - \bar{X}_{.j} + \bar{X}_{..})^2$$

Greatest stability is when

$$W = W_i^2 = 0.$$

For cotton yields stability parameters were calculated. Stability variances (σ_i^2 and S_i^2) by Shukla (1972) and ecovalence W_i by Wricke (1962) show what portion of variation related to interaction of the treatments and years are accounted by the specific variant.

Through the stability criterion (YS_i) of Kang (1993) the value of each variant was shown by simultaneously taking into account the parameter value and the stability of the variant. The value of that criterion is that by using non-parametric methods and statistical reliability of differences we obtain a combined valuation ranking variants in a descending order according to their economic value.

Table 1. Investigated variants

Herbicides						Cultivars (no GMOs)
After sowing, before emergence			During budding stage			
Herbicide	Active substance	Dose	Herbicide	Active substance	Dose	
Dual gold 960 EK + Goal 2 E	S-metolachlor + oxyfluorfen	1.2 l/ha + 1.5 l/ha	Basagran 480 SL	bentazone	1.5 l/ha	Chirpan-539
						Helius
						Trakia
						Vicky
						Philipopolis
						IPK-Veno
						Boyana
						Avangard
						Natalia
						Darmy
						Dorina
						Nelina
			Pulsar 40	imazamox	1.2 l/ha	Chirpan-539
						Helius
						Trakia
						Vicky
						Philipopolis
						IPK-Veno
						Boyana
						Avangard
						Natalia
						Darmy
						Dorina
						Nelina
			Express 50 SX	tribenuron-methyl	50 g/ha	Chirpan-539
						Helius
						Trakia
						Vicky
						Philipopolis
						IPK-Veno
						Boyana
						Avangard
						Natalia
						Darmy
						Dorina
						Nelina

To calculate these parameters, the STABLE software of Louisiana State University Agricultural Center, Baton Rouge, USA (1993) was used. The following model was applied to assess the stability of various variants in their interaction with years:

$$X_{ij} = m + N_i + Y_j + NY_{ij} + L_{ij}$$

where:

X_{ij} - grain parameter (yield, mass) of the i^{th} variant with j^{th} environment (year);
m - general mean;
N_i - effect of the i^{th} variant;
Y_j - effect of the j^{th} environment (year);
NY_{ij} - effect of interaction of the i^{th} variant with the j^{th} environment (year);
L_{ij} - error relating to the i^{th} variant in the j^{th} environment (year).

RESULTS AND DISCUSSION

On average during the study period, the herbicide Basagran 480 SL applied during cotton vegetation showed the highest phytotoxicity on fat content in seeds of the Chirpan-539 cultivar (Table 2). In it the lowest value of the indicator was measured compared to the other cultivars - 28.6%. The weakest is the effect of the herbicide Philipopolis. It has the highest fat content in seeds - 33.6%.

The Dorina cultivar was most sensitive to the herbicide Pulsar 40, the fat content in seeds was 23.1%, as compared with the cultivars Helius and Darmy which have the highest values by 28.0% and 28.4%, respectively.

In the vegetative treatment with the herbicide Express 50 WG the highest phytotoxicity on the fat content of seeds was recorded in the Nelina, Dorina and Darmy cultivars. In them the lowest values were measured 29.4%, 29.5% and 29.5%, respectively. The Helius cultivar was less affected by herbicide so the fat content in seeds was higher (32.5%).

Table 2. Fat content in cotton seeds (%)

Herbicides	Cultivars	Fat content (%) in different years			
		2013	2014	2015	Mean
Basagran	Chirpan-539	28.1	30.5	27.1	28.6
	Helius	29.0	32.0	28.0	29.7
	Trakia	31.6	31.6	30.6	31.3
	Vicky	32.0	32.5	31.0	31.8
	Philipopolis	34.3	33.1	33.3	33.6
	IPK-Veno	31.3	31.7	30.3	31.1
	Boyana	32.1	33.5	31.1	32.3
	Avangard	33.6	31.4	32.6	32.5
	Natalia	32.0	33.3	31.0	32.1
	Darmy	33.1	32.2	32.1	32.5
	Dorina	34.1	30.8	33.1	32.7
	Nelina	33.2	31.8	32.2	32.4
Pulsar	Chirpan-539	28.2	29.8	23.2	27.0
	Helius	29.2	30.7	24.2	28.0
	Trakia	25.6	30.4	20.1	25.4
	Vicky	29.0	28.9	23.3	27.0
	Philipopolis	28.7	30.3	14.0	24.3
	IPK-Veno	29.9	30.9	14.3	25.0
	Boyana	29.5	31.9	18.2	26.5
	Avangard	28.9	30.0	21.9	26.9
	Natalia	28.8	28.0	23.6	26.8
	Darmy	29.0	30.3	26.0	28.4
	Dorina	29.0	26.5	14.0	23.1
	Nelina	29.0	29.2	20.8	26.3
Express	Chirpan-539	32.1	31.8	32.1	32.0
	Helius	33.5	30.6	33.5	32.5
	Trakia	31.5	30.8	32.0	31.4
	Vicky	30.0	31.1	30.0	30.3
	Philipopolis	30.5	30.1	30.4	30.3
	IPK-Veno	30.1	31.1	30.1	30.4
	Boyana	29.9	31.3	29.8	30.3
	Avangard	32.3	29.8	32.6	31.5
	Natalia	30.7	30.0	30.7	30.5
	Darmy	29.6	29.4	29.6	29.5
	Dorina	29.7	29.2	29.7	29.5
	Nelina	29.3	30.7	28.2	29.4

LSD (%):

F.A	$p \leq 0.05 = 0.1$	$p \leq 0.01 = 0.2$	$p \leq 0.001 = 0.3$
F.B	$p \leq 0.05 = 0.1$	$p \leq 0.01 = 0.2$	$p \leq 0.001 = 0.3$
F.C	$p \leq 0.05 = 0.2$	$p \leq 0.01 = 0.3$	$p \leq 0.001 = 0.4$
AxB	$p \leq 0.05 = 0.2$	$p \leq 0.01 = 0.3$	$p \leq 0.001 = 0.4$
AxC	$p \leq 0.05 = 0.4$	$p \leq 0.01 = 0.6$	$p \leq 0.001 = 0.7$
BxC	$p \leq 0.05 = 0.4$	$p \leq 0.01 = 0.6$	$p \leq 0.001 = 0.7$
AxBxC	$p \leq 0.05 = 0.7$	$p \leq 0.01 = 1.0$	$p \leq 0.001 = 1.2$

The variance analysis of fat content in cotton seeds (Table 3) revealed that herbicides have the greatest impact on this indicator - 37.0% of the total variation. The reason for this is the phytotoxic action of some herbicides on cotton plants during vegetation. Years also have a great impact - 15.8% which is due to the different weather conditions throughout individual years of study. The degree of effect of cultivars is 1.8%. The effect of years, cultivars and herbicides is very well proven at $p \leq 0.1$. There is a proven interaction of herbicides with the conditions of years (AxB) - 24.1%, the effect of years with cultivars (AxC) is 4.1% and that of herbicides with cultivars (BxC) is 8.7%. They are very well proven at $p \leq 0.1$. There is also interaction between the three factors in the experiment (AxBxC) - 8.0%. It is also proven at $p \leq 0.1$.

Table 3. Analyses of variance for fat content

Source of variation	Degrees of freedom	Sum of squares	Influence of factor (%)	Mean squares
Total	215	3273.3	100	-
Tract of land	1	0.06	0.1	0.06
Variants	107	3258.8	99.5	30.5***
Factor A - Years	2	516.7	15.8	256.4***
Factor B - Herbicides	2	1207.7	37.0	603.8***
Factor C - Cultivars	11	59.6	1.8	5.4***
AxB	4	789.4	24.1	197.4***
AxC	22	135.6	4.1	6.2***
BxC	22	286.3	8.7	13.0***
AxBxC	44	263.5	8.0	6.0***
Pooled error	107	14.4	0.4	0.1

*$p \leq 0.05$ **$p \leq 0.01$ ***$p \leq 0.001$

By using these three stability indicators it has been found that in vegetative treatment with the herbicide Basagran 480 SL stable are the following cultivars: Trakia, Vicky, IPK-Veno and Darmy (Table 4). In treatment with Pulsar 40 stable are the cultivars Vicky and Darmy with the Express 50 WG stable are the cultivars Chirpan-539, Trakia, Philipopolis, Natalia, Darmy and Dorina. The other variants have high instability. In them Shukla's stability variance values σ_i^2 and S_i^2 and Wricke's ecovalence W_i are high and mathematically proven. Instability is mainly due to the significant differences in the fat content of seeds in these variants throughout the years of the experiment since herbicides have the strongest effect on them. In some of them there is instability of linear and non-linear type - proven values of σ_i^2 and S_i^2. In another part there is only instability of a linear type - proven value of σ_i^2 the σ_i^2 and $0S_i^2$ values are unproven.

Kang's YS_i aggregate stability criterion taking into account both the stability and the value of fat content in seeds gives negative values of cultivars Chirpan-539, Trakia, Vicky, Philipopolis, IPK-Veno, Avangard, Natalia, Dorina and Nelina, treated with Pulsar 40. They are characterized as the most unstable or the most sensitive to the herbicide regarding fat content in cotton seeds. In vegetative treatment with the herbicides Basagran 480 SL and Express 50 WG none of the cultivars received a negative evaluation. According to this criterion the most valuable in terms of fat content in cotton seeds are all cultivars with vegetative treatment with the herbicide Basagran 480 SL except Chirpan-539 and Helius. In treatment with the herbicide Express 50 VG the most valuable are Chirpan-539, Helius, Trakia, Vicky, Philipopolis, IPK-Veno, Boyana, Avangard and Natalia. They combine high values and high stability with regard to fat content in seeds throughout the years. In vegetative treatment with Pulsar 40 none of the cultivars was highly rated.

Table 4. Stability parameters for the variants for fat content with relation to years

Herbicides	Cultivars	$\bar{x}$	σ_i^2	S_i^2	W_i	YS_i
Basagran	Chirpan-539	28.6	2.7**	5.1**	6.1	2
	Helius	29.7	3.9**	8.1**	8.2	10
	Trakia	31.3	3.3**	-0.07	7.1	19+
	Vicky	31.8	2.5**	0.08	5.6	22+
	Philipopolis	33.6	6.6**	1.5**	13.3	31+
	IPK-Veno	31.1	2.6**	0.01	5.9	18+
	Boyana	32.3	1.9**	1.6**	4.6	25+
	Avangard	32.5	10.9**	4.9**	21.5	27+
	Natalia	32.1	2.0**	1.3**	4.7	24+
	Darmy	32.5	2.9**	-0.06	6.1	30+
	Dorina	32.7	17.2**	11.0**	33.5	29+
	Nelina	32.4	7.3**	2.1**	14.8	26+
Pulsar	Chirpan-539	27.0	4.9**	1.2**	10.1	-2
	Helius	28.0	4.6**	1.0**	9.5	0
	Trakia	25.4	25.3**	17.9**	48.8	-7
	Vicky	27.0	3.5**	0.3	7.5	-2
	Philipopolis	24.3	105.6**	0.02	200.4	-9
	IPK-Veno	25.0	115.6**	0.04	219.2	-8
	Boyana	26.5	61.7**	1.9**	117.5	-5
	Avangard	26.9	12.7**	0.2	24.8	-3
	Natalia	26.8	1.7**	1.4**	4.2	-4
	Darmy	28.4	0.1	0.9**	1.2	9
	Dorina	23.1	80.8**	13.9**	15.35	-10
	Nelina	26.3	16.8**	0.08	32.8	-6
Express	Chirpan-539	32.0	8.0**	0.09	16.1	23+
	Helius	32.5	20.7**	8.1**	40.1	27+
	Trakia	31.4	12.1**	0.3	23.8	20+
	Vicky	30.3	5.1**	1.1**	10.7	15+
	Philipopolis	30.3	7.9**	0.08	15.8	13+
	IPK-Veno	30.4	5.3**	0.9*	10.9	16+
	Boyana	30.3	4.6**	1.8**	9.6	13+
	Avangard	31.5	20.8**	6.3**	40.2	21+
	Natalia	30.5	9.4**	0.4	18.6	17+
	Darmy	29.5	7.7**	-0.03	15.6	6
	Dorina	29.5	8.7**	0.2	17.3	7
	Nelina	29.4	1.8**	1.6**	4.2	5

Genetically improved cultivars and hybrids resistant to herbicides are widely used in many field crops in the world. These cultivars and hybrids are not GMOs and they are used within the territory of the all European Union. In Bulgaria with their help are solved the problems of secondary weed infestation in some field crops such as sunflower, maize, canola (Delchev, 2018 and 2020).

Secondary weed infestation with annual and perennial broadleaf weeds is a huge problem for the cotton fields in Bulgaria. To combat these weeds are made 3-4 hands hoeing with hoes. They are very heavy, labor intensive, and greatly increase the cost of cotton production. Until now, this has made Bulgarian cotton production unprofitable and it could not compete with cheap GMO cotton produced in the major cotton-producing countries outside Europe. We emptied significant number of studies to find cotton cultivars resistant to foliar-applied antibroadleaf herbicides. For the first time in the world cotton cultivars resistant to herbicides Basagran 480 SL (bentazone), Pulsar 40 (imazamox) and Express 50 VG (tribenuron-methyl) have been established. These cultivars are Bulgarian and have been created in the Field Crops Institute, Chirpan. They produce high and stable yields of raw cotton, cotton fiber and cotton seeds over the years (Barakova and Delchev, 2016;

Barakova, 2017). The use of the herbicides bentazone, imazamox and tribenuron-methyl in cotton provides complete control of late spring annual broadleaf weeds *Xanthium strumarium* L., *Amaranthus retroflexus* L., *Amaranthus albus* L., *Amaranthus blifoides* W., *Chenopodium album* L., *Solanum nigrum* L., *Datura stramonium* L., *Polygonum aviculare* L., *Abutilon teophrasti* Medic., *Portulaca oleracea* L., *Polygonum aviculare* L., *Hibiscum trionum* L., *Tribulus terrestris* L. The herbicides imazamox and tribenuron-methyl also provide complete control of the perennial broadleaf weeds *Cirsium arvense* Scop. and *Convolvulus arvensis* L. (Barakova, 2017).

The obtained results are of great importance not only for Bulgaria but also for the other countries producing cotton in the European Union - Greece, Italy, Spain and Portugal, who cannot use genetically modified cotton cultivars. This requires a study of the quality indicators of the fiber and seeds of these genetically improved cultivars resistant to herbicides. The herbicide Basagran 480 SL, applied during cotton vegetation has the highest phytotoxicity on fat content in cotton seeds of Chirpan-539 cultivar. The highest fat content is reported for the Philipopolis cultivar. The herbicide Pulsar 40 has the strongest phytotoxic effect on fat content of cotton seeds of the Dorina cultivar. Cultivars Helius and Darmy have the highest fat content in seeds. In vegetative treatment with the herbicide Express 50 WG, the highest phytotoxicity on the fat content in seeds was recorded in cultivars Nelina, Dorina and Darmy. The weakest is the effect of the herbicide on that indicator in Helius cultivar. For the first time in the world it has been established that in the vegetative treatment with herbicides the most valuable with regard to cottonseed fat content are all cultivars treated with Basagran 480 SL, except Chirpan-539 and Helius. This shows that herbicides affected differently as the yield of cotton and the amount of fat in cottonseed. This should be taken into account when these seeds are used to produce cottonseed oil. Herbicides that reduce the fat content should not be used in the respective cultivars.

The analysis of variance for fat content showed that the herbicidal action strongly depends on weather conditions during the vegetation period. These are mainly temperature and rainfall after the herbicide treatment. Cotton cultivars also react differently when treated with herbicides Basagran, Pulsar and Express in different years.

Based on the proven interactions of herbicide x year and cultivar x year the stability of manifestation of each variant has been assessed with regard to fat content in cotton seeds. Shukla's stability variances σ_i^2 and S_i^2, Wricke's ecovalence W_i and the Kang's YS_i stability criterion have also been calculated.

Shukla's stability variances (σ_i^2 and S_i^2) which take into account both linear and non-linear interactions uniquely assess the stability of variants. The variants with lower values are considered to be more stable because they interact less with environmental conditions. The negative values of the indicators σ_i^2 and S_i^2 are assumed to be 0. In reliably high values of either parameter - σ_i^2 or S_i^2 the variants are considered to be unstable. With Wricke's ecovalence W_i the higher the values of the indicator the more unstable the relevant variant.

In order to make an overall assessment of the effectiveness of each herbicide both its effect on fat content and its stability - reaction of cotton cultivars to it throughout the years - should be taken into account. Very valuable information about the technological value of variants is given by Kang's YS_i criterion for simultaneous evaluation of seed fat content and stability based on the reliability of differences in the fat content and the variance of interaction with the environment. The value of this criterion is that by using non-parametric methods and statistical proof of differences. We obtain a generalized assessment ranking variants in descending order according to their economic value.

It has been established for the first time that from the point of view of growing technology with vegetative treatment with the herbicide Express 50 WG, the most valuable are the cultivars Chirpan-539, Helius, Trakia, Vicky, Philipopolis, IPK-Veno, Boyana, Avangard, Natalia.

None of the cultivars is highly rated when treated with the herbicide Pulsar 40 with regard to fat content in seeds.

CONCLUSIONS

For the first time in the world it has been established that in the vegetative treatment with herbicides the most valuable with regard to cotton seed fat content are all cultivars treated with Basagran 480 SL, except Chirpan-539 and Helius.

It has been established for the first time that from the point of view of growing technology with vegetative treatment with the herbicide Express 50 WG, the most valuable are the cultivars Chirpan-539, Helius, Trakia, Vicky, Philipopolis, IPK-Veno, Boyana, Avangard, Natalia.

None of the cultivars is highly rated when treated with the herbicide Pulsar 40 with regard to fat content in seeds.

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