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EFFECT OF SOME HERBICIDES AND THEIR MIXTURES WITH GROWTH REGULATOR AND FOLIAR FERTILIZER ON PROTEIN CONTENT IN COTTON (*Gossypium hirsutum* L.) SEEDS

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ABSTRACT

For the first time in the world we established that the highest protein content in seeds of cultivar Helius was obtained with vegetative treatment with herbicides Goal 2 E, Linuron 45 SC, Wing P and Merlin 750 WG. The highest protein content in seeds of cultivar Darmy was obtained with vegetative treatment with herbicides Goal 2 E, Wing P and Basagran 480 SL. The highest phytotoxicity on protein content in seeds of cultivar Helius was shown by the herbicide Basagran 480 SL and all herbicide combinations with the growth regulator Amalgerol and the foliar fertilizer Lactofol O. The strongest phytotoxic effects on seeds of cultivar Darmy was produced by the herbicides Linuron 45 SC and Merlin 750 WG and herbicide mixture Wing P + Lactofol O. For the first time, we established that, from the point of view of the technology for cotton growing of cultivar Helius, the most valuable are tank mixtures Goal 2 E + Amalgerol, Linuron 45 SC + Amalgerol, Goal 2 E + Lactofol O, as well as the single use of herbicides Goal 2 E, Linuron 45 SC, Wing P and Merlin 750 WG. Technologically the most valuable in cotton cultivar Darmy were herbicide combinations Goal 2 E + Amalgerol, Wing P + Amalgerol, Merlin 750 WG + Amalgerol, Basagran 480 SL + Amalgerol, Goal 2 E + Lactofol O, Linuron 45 SC + Lactofol O, Merlin 750 WG + Lactofol O and Basagrain 480 SL + Lactofol O, and the herbicides Goal 2 E, Wing P and Basagran 480 SL.

Keywords: cotton, protein content, herbicides, foliar fertilizer, growth regulator.

INTRODUCTION

Cotton is the most valuable fibre crop and one of the most valuable oil crops in the world. Cotton seeds are rich in protein (40-43%).

A problem in modern cotton growing is the secondary weed infestation in the crop (Boz, 2000; Nikolova, 2001; Economou, 2005; Gozgu and Uludag, 2005; Gaylon et al., 2015; Spielman et al., 2015). By applying anti-broadleaf herbicides on cotton areas to control weeds during the vegetation phytotoxicity is frequently manifested (Barakova and Delchev, 2016; Barakova, 2017).

Worldwide, a great deal of research has been done on protein and fat content in cotton seeds, their qualities and use (Ataullaev et al., 1982; Ashok, 2006; Constantine, 2007; Saldzhiev et al., 2008; Uzunova, 2008; Saldzhieva et al., 2009). In scientific literature there is yet no information whether herbicide treatment during cotton vegetation affects protein content in cotton seeds.

The objective of this study was to investigate the effects of vegetative treatment with some herbicides and their tank mixtures with growth regulator and foliar fertilizer on protein content in cotton (*Gossypium hirsutum* L.) seeds.

MATERIAL AND METHODS

In the period 2013-2015 a field experiment was carried out in the experimental field of the Field Crops Institute, Chirpan, under non-irrigated conditions on a leached vertisol soil type with two Bulgarian cotton cultivars - Helius and Darmy (*Gossypium hirsutum* L.). The experiment was designed according to the block method in 4 replications (Dimova et al., 1999; Shannin, 1977) with a plot size of 20 m^2.

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In both cultivars five herbicides were studied - Goal 2 E, Linuron 45 SC, Wing P, Merlin 750 WG and Basagran 480 SL. They were applied both individually and in combinations of each of them with the growth regulator Amalgerol and the foliar fertilizer Lactofol O, during bud formation stage of cotton. The active substances of the herbicides and the treatment doses are given in Table 1. These herbicides were applied against the background of the herbicide combination Dual gold 960 EC 1.2 l/ha + Goal 2 E – 1.2 l/ha applied after sowing pre-emergence for control of primary weed infestation in cotton. Spraying was done with hand back sprayer with working solution of 300 l ha^{-1}. Weeds in the hoeing control were removed by hoe - 3 times during cotton vegetation.

Table 1. Investigated herbicides and herbicide combinations

№	Variants	Active substance	Doses
1	Not treated control	-	-
2	Hoeing control	-	-
3	Goal 2 E	oxifluorfen	800 ml ha^{-1}
4	Linuron 45 SC	linuron	2 l ha^{-1}
5	Wing-P	pendimethalin + dimethenamid	4 l ha^{-1}
6	Merlin 750 WG	izoxaflutole	50 g ha^{-1}
7	Basagran 480 SL	bentazone	1.5 l ha^{-1}
8	Amalgerol	growth regulator	5 l ha^{-1}
9	Goal 2 E + Amalgerol	oxifluorfen + growth regulator	800 ml ha^{-1} + 5 l ha^{-1}
10	Linuron 45 SC + Amalgerol	linuron + growth regulator	2 l ha^{-1} + 5 l ha^{-1}
11	Wing-P + Amalgerol	pendimethalin + dimethenamid + growth regulator	4 l ha^{-1} + 5 l ha^{-1}
12	Merlin 750 WG + Amalgerol	izoxaflutole + growth regulator	50 g ha^{-1} + 5 l ha^{-1}
13	Basagran 480 SL + Amalgerol	bentazone + growth regulator	1.5 l ha^{-1} + 5 l ha^{-1}
14	Lactofol O	foliar fertilizer	8 l ha^{-1}
15	Goal 2 E + Lactofol O	oxifluorfen + foliar fertilizer	800 ml ha^{-1} + 8 l ha^{-1}
16	Linuron 45 SC + Lactofol O	linuron + foliar fertilizer	2 l ha^{-1} + 8 l ha^{-1}
17	Wing-P + Lactofol O	pendimethalin + dimethenamid + foliar fertilizer	4 l ha^{-1} + 8 l ha^{-1}
18	Merlin 750 WG + Lactofol O	izoxaflutole + foliar fertilizer	50 g ha^{-1} + 8 l ha^{-1}
19	Basagran 480 SL + Lactofol O	bentazone + foliar fertilizer	1.5 l ha^{-1} + 8 l ha^{-1}

Protein content in cotton seeds was determined by the Kjeldahl method - SR ISO 5983. The seeds were taken from cotton treated during vegetation with the relevant herbicides and herbicide tank mixtures.

Statistical evaluation to estimate the representativeness and reliable effect of the studied parameters was applied through dispersion analysis and Fischer's parametric criterion F (Shannin, 1977; Barov, 1982). In the variance analysis the ANOVA123 software was used for calculation (Lidanski, 1988).

The selectivity of herbicides was established through their effect on grain yield and the following variances were calculated:

Shukla (1972) stability variance (σ_i^2)

$$Sh-\sigma_i^2 = \left[1/(e-1)(t-1)(t-2)\right] \times \left[t(t-1)\sum_{j=1}^{s}(u_{ij}-\bar{u}_i)^2 - \sum_{i=1}^{t}\sum_{j=1}^{s}(u_{ij}-\bar{u}_i)^2\right]$$

where:

$u_{ij} = X_{ij} - \bar{X}_{.j}$

X_{ij} = observed trait value of i^{th} cultivar in j^{th} environment;

$\bar{X}_{.j}$ = mean of all cultivars in j^{th} environment;

$$\bar{u}_{i.} = \sum_{j=1}^{m} u_{ij}/e$$

e = number of environments;

t = number of cultivars.

In this study, calculation of adjusted stability variance (Sh-Si^2) was necessary, because the heterogeneity term was significant ($p<0.01$).

The stability statistic Sh-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 and $\bar{X}_{..}$ = mean of all cultivars across all environments, using the following equation (Shukla, 1972):

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

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 protein content 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 preparations 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.

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 three years of study, the herbicide Basagran 480 SL and some herbicide combinations with the growth regulator Amalgerol and the foliar fertilizer Lactofol O had adverse effect on protein content in seeds of cultivar Helius (Table 2). In them values lower than the economic control were reported – 38.2%. The lowest value of the indicator was measured in the herbicide combination Merlin 750 WG + Lactofol O – 32.8%. The single use of the herbicides Goal 2E, Linuron 45 SC, Wing P and Merlin 750 WG did not affect protein content in seeds of cultivar Helius.

Herbicides Merlin 750 WG and Linuron 45 SC and the herbicide combinations – Wing P + Amalgerol and Wing P + Lactofol O had phytotoxic action on protein content in seeds of cultivar Darmy 38.3% compared to that of the economic control. The single use of the other herbicides and all other herbicide mixtures did not affect the indicator. The highest protein content was recorded for the herbicides Goal 2 E – 39.4%, Wing P – 39.4% and Basagran 480 SL – 39.8%. Herbicide mixtures Goal 2 E + Amalgerol and Merlin 750 WG + Amalgerol recorded the highest values, 40.0% and 40.4%, respectively.

Table 2. Protein content (%) in cotton seeds after vegetation treatment with herbicides (2013-2015)

Cultivars	Variants	2013	2014	2015	Mean
Helius	Not treated control	31.0	30.0	32.0	31.0
	Hoeing control	37.1	38.5	39.1	38.2
	Goal 2 E	38.2	37.5	39.4	38.3
	Linuron 45 SC	38.0	37.5	40.0	38.5
	Wing-P	38.2	37.4	39.2	38.3
	Merlin 750 WG	38.2	37.2	39.2	38.2
	Basagran 480 SL	36.7	35.8	37.7	36.7
	Amalgerol	36.1	35.1	37.1	36.1
	Goal 2 E + Amalgerol	38.1	37.1	38.6	37.9
	Linuron 45 SC + Amalgerol	38.2	37.2	38.7	38.0
	Wing-P + Amalgerol	36.0	36.1	37.0	36.3
	Merlin 750 WG + Amalgerol	36.2	36.3	37.2	36.5
	Basagran 480 SL + Amalgerol	35.5	35.5	36.0	35.7
	Lactofol O	38.1	37.1	38.1	37.7
	Goal 2 E + Lactofol O	37.2	37.2	38.2	37.5
	Linuron 45 SC + Lactofol O	35.3	35.3	36.3	35.6
	Wing-P + Lactofol O	35.1	36.1	35.1	35.4
	Merlin 750 WG + Lactofol O	33.1	33.2	32.1	32.8
	Basagran 480 SL + Lactofol O	35.9	35.9	36.9	36.2
Darmy	Not treated control	31.3	31.3	32.4	31.7
	Hoeing control	37.1	39.1	38.6	38.3
	Goal 2 E	38.1	41.1	39.1	39.4
	Linuron 45 SC	38.5	32.5	39.9	36.9
	Wing-P	39.0	39.5	39.5	39.4
	Merlin 750 WG	37.5	35.5	38.5	37.1
	Basagran 480 SL	38.8	40.3	40.3	39.8
	Amalgerol	36.7	36.7	37.7	37.0
	Goal 2 E + Amalgerol	39.4	40.4	40.4	40.0
	Linuron 45 SC + Amalgerol	40.5	37.0	39.0	38.8
	Wing-P + Amalgerol	37.4	38.4	38.4	38.0
	Merlin 750 WG + Amalgerol	39.1	41.1	41.1	40.4
	Basagran 480 SL + Amalgerol	39.5	40.0	40.0	39.9
	Lactofol O	38.6	38.6	39.1	38.8
	Goal 2 E + Lactofol O	39.5	39.5	40.5	39.9
	Linuron 45 SC + Lactofol O	39.6	39.6	40.1	39.8
	Wing-P + Lactofol O	35.4	35.4	36.4	35.7
	Merlin 750 WG + Lactofol O	37.9	39.9	39.9	39.3
	Basagran 480 SL + Lactofol O	38.5	38.5	39.5	38.8

LSD, %:

F.A	p≤5%=0.2	p≤1%=0.3	p≤0.1%=0.4
F.B	p≤5%=0.1	p≤1%=0.2	p≤0.1%=0.3
F.C	p≤5%=0.4	p≤1%=0.5	p≤0.1%=0.7
AxB	p≤5%=0.2	p≤1%=0.3	p≤0.1%=0.4
AxC	p≤5%=0.7	p≤1%=0.9	p≤0.1%=1.1
BxC	p≤5%=0.5	p≤1%=0.7	p≤0.1%=0.9
AxBxC	p≤5%=1.0	p≤1%=1.3	p≤0.1%=1.6

The variance analysis with regard to protein content in cotton seeds (Table 3) established that herbicides and herbicide combinations had the greatest impact on that indicator - 53.0% of the total variation. The reason for this is the phytotoxic action of some of the herbicides on cotton plants during vegetation. Cultivars also had great influence - 14.3%, which was due to the different genetic origin of the two cotton cultivars - Helius and Darmy. The effect of years represented 3.7% of total variation. The effect of years, cultivars and herbicides was significant at p≤0.1. There was significant interaction of cultivars with the conditions of years (AxB) - 0.2%, significant at p≤5. The effect of interaction between years and herbicides (AxC) was 5.9% and that of cultivars with preparations (BxC) - 16.6%. They were very significant at p≤0.1. There was also an interaction among the three factors in the experiment (AxBxC) – representing 4.1%, also significant at p≤0.1.

Table 3. Analyses of variance for protein content

Source of variation	Degrees of freedom	Sum of squares	Influence of factor (%)	Mean squares
Total	455	1256.7	100	-
Tract of land	3	0.1	0.1	0.1
Variants	113	1230.6	97.8	10.9***
Factor A - Years	2	46.0	3.7	23.0***
Factor B - Cultivars	1	180.2	14.3	18.02***
Factor C - Herbicides	18	668.9	53.0	37.2***
AxB	2	2.0	0.2	1.0*
AxC	36	73.8	5.9	2.1***
BxC	18	208.8	16.6	11.6***
AxBxC	36	50.9	4.1	1.4***
Pooled error	339	26.1	2.1	0.2

*p≤5%; **p≤1%; ***p≤0.1%.

Based on the significant interactions, preparation x year and cultivar x year, the stability of manifestations of each variant with respect to protein content in cotton seeds was evaluated (Table 4). Shukla's stability variances σ_i^2 and S_i^2, Wricke's ecovalence Wi and Kang's stability criterion YS_i were 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 the variants. These variants, which show lower values, are considered to be more stable, because they interact less with the environmental conditions. The negative values of the indicators σ_i^2 and S_i^2 are assumed to be 0. With reliably high values of either parameter - σ_i^2 or S_i^2, the variants are considered unstable. With Wricke's ecovalence W_i, the higher the values of the indicator, the more unstable the relevant variant.

By using these three stability parameters we concluded that, in cultivar Helius the effects of herbicides Wing P, Merlin 750 WG, Basagran 480 SL, all herbicide combinations with the growth regulator Amalgerol, herbicide mixtures Goal + Lactofol O and Basagran 480 SL + Lactofol O, as well as the growth regulator and the foliar fertilizer, were stable. In cultivar Darmy, the effects of herbicide Wing P, herbicide combinations Basagran 480 SL + Amalgerol, Goal 2 E + Lactofol O, Linuron 45 SC + Lactofol O, Wing P + Lactofol O, Basagran 480 SL + Lactofol O were stable. The other variants were highly unstable. In them the values of the Shukla σ_i^2 and S_i^2 stability variances and Wricke's ecovalence Wi were high and mathematically significant. Instability was mainly due to the significant differences in protein content in cotton seed in these variants throughout the years of the experiment, since herbicides and herbicide combinations had the strongest effect on them. In some of them there was instability of linear and non-linear type - significant values of σ_i^2 and S_i^2. On the other hand, there was only linear type of instability - significant value of σ_i^2, whereas the S_i^2 values are not significant.

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

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

Cultivars	Variants	$\bar{x}$	σ_i^2	S_i^2	W_i	YS_i
Helius	Not treated control	31.0	0.6	0.5	1.2	-2
	Hoeing control	38.2	1.4**	2.7**	2.7	17+
	Goal 2 E	38.3	0.8**	0.4	1.6	22+
	Linuron 45 SC	38.5	1.3**	-0.05	2.7	22+
	Wing-P	38.3	0.4	0.2	0.8	26+
	Merlin 750 WG	38.2	0.6	0.5	1.2	24+
	Basagran 480 SL	36.7	0.5	0.3	1.0	10
	Amalgerol	36.1	0.6	0.5	1.2	4
	Goal 2 E + Amalgerol	37.9	0.3	0.6	0.7	19+
	Linuron 45 SC + Amalgerol	38.0	0.3	0.6	0.7	21+
	Wing-P + Amalgerol	36.3	-0.09	0.02	0.08	7
	Merlin 750 WG + Amalgerol	36.5	-0.09	0.02	0.08	9
	Basagran 480 SL + Amalgerol	35.7	0.05	-0.05	0.2	2
	Lactofol O	37.7	0.4	0.8	0.9	18+
	Goal 2 E + Lactofol O	37.5	-0.02	-0.02	0.05	17+
	Linuron 45 SC + Lactofol O	35.6	2.0**	3.6**	3.9	-2
	Wing-P + Lactofol O	35.4	2.0**	0.8	3.9	-7
	Merlin 750 WG + Lactofol O	32.8	2.6**	-0.05	5.0	-8
	Basagran 480 SL + Lactofol O	36.2	-0.02	-0.02	0.05	5
Darmy	no treated control	31.7	0.04	0.01	0.09	-1
	hoeing control	38.3	2.5**	4.5**	4.9	18+
	Goal 2 E	39.4	6.6**	8.6**	12.5	27+
	Linuron 45 SC	36.9	26.7**	27.1**	50.6	3
	Wing-P	39.4	0.4	0.2	1.0	34+
	Merlin 750 WG	37.1	2.6**	2.6**	5.1	6
	Basagran 480 SL	39.8	1.4**	2.7**	2.8	29+
	Amalgerol	37.0	-0.02	-0.02	0.05	13
	Goal 2 E + Amalgerol	40.0	0.8*	1.2*	1.5	26+
	Linuron 45 SC + Amalgerol	38.8	10.6**	15.1**	20.2	15
	Wing-P + Amalgerol	38.0	0.8*	1.2*	1.5	18+
	Merlin 750 WG + Amalgerol	40.4	2.4**	4.9**	4.7	33+
	Basagran 480 SL + Amalgerol	39.9	0.5	0.2	1.0	38+
	Lactofol O	38.8	0.05	-0.05	0.2	31+
	Goal 2 E + Lactofol O	39.9	-0.02	-0.02	0.05	38+
	Linuron 45 SC + Lactofol O	39.8	0.05	-0.05	0.2	36+
	Wing-P + Lactofol O	35.7	-0.02	-0.02	0.05	3
	Merlin 750 WG + Lactofol O	39.3	2.4**	4.9**	4.7	25+
	Basagran 480 SL + Lactofol O	38.8	-0.02	-0.02	0.05	32+

Summarizing Kang's stability criterion YSi, taking into account both stability and protein value in cotton seeds, gives negative assessment for cultivar Helius treated with herbicide combinations Linuron 45 SC + Lactofol O, Wing P + Lactofol O and Merlin 750 WG + Lactofol O. It is characterized as the most unstable or the most sensitive to herbicides in terms of protein content in cotton seeds. In cultivar Darmy, none of the herbicides and herbicide mixtures had negative assessment. According to that criterion, technologically the most valuable in cultivar Helius are herbicide combinations Goal 2 E + Amalgerol, Linuron 45 SC + Amalgerol, Goal + Lactofol O, and the single use of herbicides Goal 2 E, Linuron 45 SC, Wing P and Merlin 750 WG. In cultivar Darmy, technologically the most valuable are the following herbicides: Goal 2E, Wing P and Basagran 480 SL, tank combinations: Goal 2 E + Amalgerol, Wing P + Amalgerol, Merlin 750 WG + Amalgerol, Basagran 480 SL + Amalgerol + Goal 2 E + Lactofol O, Linuron 45 SC + Lactofol O, Merlin 750 WG + Lactofol O, Basagran 480 SL + Lactofol O. They combine high values and high stability with regard to protein content in cotton seeds in different years. Foliar fertilizer Lactofol O in both cotton cultivars had positive rating according to this criterion.

CONCLUSIONS

For the first time in the world we established that the highest protein content in seeds of cultivar Helius was obtained in vegetative treatment with herbicides Goal 2 E, Linuron 45 SC, Wing P and Merlin 750 WG.

The highest is the protein content in seeds of cultivar Darmy was obtained with vegetative treatment with herbicides Goal 2 E, Wing P and Basagran 480 SL.

The highest phytotoxicity on protein content in seeds of cultivar Helius was shown by the herbicide Basagran 480 SL and all herbicide combinations with the growth regulator Amalgerol and the foliar fertilizer Lactofol O.

The strongest phytotoxic effects on seeds of cultivar Darmy had the herbicides Linuron 45 SC and Merlin 750 WG and herbicide mixture Wing P + Lactofol O.

For the first time, we established that, technologically the most valuable crop management solutions for cultivar Helius, were tank mixtures Goal 2 E + Amalgerol, Linuron 45 SC + Amalgerol, Goal 2 E + Lactofol O, as well as the single use of herbicides Goal 2 E, Linuron 45 SC, Wing P and Merlin 750 WG.

For the cultivar Darmy, technologically the most valuable solution were herbicide combinations Goal 2 E + Amalgerol, Wing P + Amalgerol, Merlin 750 WG + Amalgerol, Basagran 480 SL + Amalgerol, Goal 2 E + Lactofol O, Linuron 45 SC + Lactofol O, Merlin 750 WG + Lactofol O and Basagran 480 SL + Lactofol O, as well as the herbicides Goal 2 E, Wing P and Basagran 480 SL.

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