Study on biostimulant application at oilseed rape damaged by simulated herbicide drift

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Abstract

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Herbicides are powerful means for weed control in modern agriculture. However, in some cases they can negatively affect the crops, for example after herbicide drift. One suitable approach, aimed to improve recovery of herbicide-damaged crops, is the therapeutic biostimulant application. The aim of our study was to evaluate the potential of the plant biostimulant Amino Expert® to ameliorate recovering of florasulam + aminopyralid-potassium (Derby® Super) – damaged oilseed rape plants. The experiment included five treatments: 1. Untreated plot, accepted as a control. Treatments 2 and 3 represented Derby® Super 100% (of the registered rate) and Derby® Super 20% respectively. The treatments 4 and 5 included two applications – first by the herbicide and second – by the biostimulant four days later. The observations showed that the herbicide drift caused negative effect on oilseed rape plants distinguished by yellowing and growth retardation, which was visible to the beginning of flowering. On the contrary, the plants received an additional therapeutic application by Amino Expert® Impuls recovered faster and finally developed a higher silique number plant⁻¹ as well as biological yield. The physiological parameters such as photosynthetic rate and pigments content also confirmed the ameliorative effect of the plant biostimulant application.

Keywords: Brassica napus L.; herbicide drift; phytotoxicity; plant biostimulant

Introduction

Herbicides are unique in that they are designed to destroy plants. Sufficiently high doses will kill both crops and weeds, while low rates have no effect upon crops or weeds. The action of an herbicide is usually determined by its chemical and physical properties, its effect on plant metabolism, the plant itself as well as the environmental conditions (Streibig, 2003).

Herbicides are "foreign" to the plants substances – xenobiotics. Plants respond to invasion of foreign substances by expression of various mechanisms of detoxification. When the tolerance of cultivated plants to the absorbed herbicide is not enough to destroy the crop, the result is herbicide stress leading to various structural and functional distortions. Herbicide phytotoxicity can also occur with long-term effects and herbicide drift on non-target crops (Vischetti et al., 2002).

Herbicide drift is the movement of herbicide from the target area to areas where herbicide solution is not intended to go. There are a great number of factors that can lead to spray drift: spray particle size; method of application; distance between nozzle and target (boom height); relative humidity and temperature; wind direction; spray pressure; spray angle; air movement around aircraft; etc. (Dexter, 1993).

Numerous authors are working on the problems caused by herbicide drift of different herbicide molecules (Snoo & Van der Poll, 1999; Ellis & Griffin, 2002; Al-Khatib et al., 2003; Londo et al., 2010; Colquhoun et al., 2014; Mohseni-Moghadam & Doohan, 2015; Smith et al., 2017). The development of plants that are under stress conditions such as drought, nutrients shortage, insect-caused damages and other stress factors, including herbicides, could be improved by biostimulant application (Khan et al., 2011; Jablonkai, 2013; Calvo et al., 2014; Harizanova et al., 2014; Nardi et al., 2016; Harizanova & Koleva-Valkova, 2019; Harizanova, et al., 2019).

The available information for the effect of biostimulants on plants damaged by herbicide drift is very poor. Therefore the aim of the current study is to evaluate the medicative effect of biostimulant application on herbicide-damaged oilseed rape plants.

Materials and Methods

The experiment was situated in the experimental field of the base for training and implementation of the Agricultural University of Plovdiv, Bulgaria. The trial was conducted by the randomized block design in 3 replications. The size of the experimental plot was 10 m^2 . The study was conducted during three vegetation periods – 2017/2018, 2018/2019 and 2019/2020.

The oilseed rape (*Brassica napus* L.) hybrid was PT 228 CL (www.corteva.bg, 2020). The hybrid is bred to be grown by the Clearfield[®] technology.

The studied physiological parameters (in 2018) were:

• Photosynthetic Pigments Content

Photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and total carotenoids) were extracted in 80% (v/v) acetone, measured spectrophotometrically and calculated according to Lichtenthaler's formulae (1987).

• Leaf Gas Exchange Analysis

Leaf gas exchange (net photosynthetic rate – A was measured on the fully developed leaves with an open photosynthetic system LCpro+ (Analytical Development Company Ltd., Hoddesdon, England).

The study included the following treatments:

- 1. Untreated control;
- 2. Derby[®] Super 100 % 33.0 g ha⁻¹;
- 3. Derby[®] Super 20 % 6.6 g ha⁻¹;
- 4. Derby[®] Super 100 % 33.0 g ha⁻¹ + Amino Expert[®] Impuls – 3.0 l ha⁻¹;
- 5. Derby[®] Super 20 % 6.6 g ha⁻¹ + Amino Expert[®] Impuls 3.0 l ha⁻¹.

Treatments 2 and 3 represented Derby[®] Super 33,0 g ha⁻¹ (100% of the registered rate) and Derby[®] Super 6,6 g ha⁻¹ (20% of the registered rate) respectively. The herbicide drift simulation was performed in BBCH 12-13 (rosette stage). Treatments 4 and 5 included two applications – first by the

herbicide and second – by the mentioned biostimulant in a rate of $3.0 \ l \ ha^{-1} \ 4 \ days$ later.

The herbicide product Derby[®] Super is registered for broadleaf weeds control in winter wheat at rates from 25 to 33 g ha⁻¹ (https://preparati.info).

The products with biostimulant mode of action used in the study was Amino Expert[®] Impuls (Amino acids -5.00% (free amino acids -4.43%); Macroelements: N -2.53%; MgO -0.50%; SO₃ -4.02%; Phytohormones -0.0003%; Organic substances and natural adhesives: 73.96\%; Microelements: B -0.52; Cu -0.39%; Fe -0.38%; Mn -0.38%; Mo -0.08%; Zn -0.78%) (http://ecofol.com, 2018).

Visual phytotoxicity on the 7th day after herbicide application was determined using the 9-score phytotoxicity scale of EWRS (European Weed Research Society):

- No effect;
- Very slight effects;
- Very slight effects; some stunting and yellowing just visible;
- Slight effects; stunting and yellowing, effects reversible;
- Substantial chlorosis and or stunting, most effects probably reversible;
- Strong chlorosis/stunting; thinning of stand;
- Increasing severity of damage;
- Increasing severity of damage;
- Increasing severity of damage;
- Total loss of plants and yield.
- The following parameters were studied:
- Silique number plant⁻¹;
- Productivity of the plants (t ha⁻¹);
- Absolute seed mass of 1000 seeds (g) and Hectoliter seed mass (kg) (Tonev et al., 2018).

For keeping the trial area free of weeds, Butisan[®] 400 SC (400 g/l metazachlor) in rate of 2.0 l ha⁻¹ after sowing before germination was applied. The existing and not controlled weeds were removed manually.

In the three years of the study predecessor of the oilseed rape was winter wheat. Planting density of the oilseed rape was 330.000 plants ha⁻¹.

On the whole experimental field combined fertilization with 250 kg ha⁻¹ with N:P:K (15:15:15), followed by deep ploughing was done. Before sowing of the crop, disking on the depth of 15 cm and two harrowings on 8 cm of depth as well as spring dressing with 250 kg ha⁻¹ NH_4NO_3 was also performed.

For statistical evaluation of the obtained data the Duncan's multiple range test was used by the software program SPSS 17.

Results and Discussion

The visual evaluation of the phytotoxicity caused by the herbicide drift is presented of Figure 1. The evaluated phytotoxicity is higher in the first year of the study. This is probably due the meteorological conditions in this period of the study. According to Tonev et al. (2007), the agro-ecological conditions at the time of herbicide application as well as in the first several days after are influencing the retention and the absorption of the foliar herbicides.

The visual phytotoxicity symptoms caused by the herbicide were the highest for the treatment that received the registered rate of Derby Super (treatment 2) in the three years of the study. For this treatment the phytotoxicity score was 3 (in 2018) and 2 (in 2019 and 2020). The observed damage symptoms were yellowing of the fully developed leaves and anthocyanin discoloration of the young leaves. Also, stunning and growth retardation was observed for this treatment.

For treatment 3 (Derby Super -6.6 g ha⁻¹) the phytotoxicity was lower - score 2 (in 2018) and 1 (in 2019 and 2020). For the treatments with applied biostimulant, the phytotoxicity score was determined as 1 (very slight effects). The visual damage symptoms disappeared in time.

For better plant performance evaluation we measured the mean physiological parameters of the plants such as content of photosynthetic pigments and leaf gas exchange of the oil-



Fig. 1. Visual phytotoxicity by EWRS (scores)

Table 1. Physiologica	l parameters (of oil seed	rape plants	(2018)
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seed rape plants during the vegetation (Table 1). The dose of Derby Super 100% is inhibiting the synthesis of photosynthetic pigments which leads to significant retardation (23%) of photosynthetic rate.

The 20% of the herbicide dose is leading to slight decrease of the pigments content, but in much lower extend, where the gas exchange of the treated plants (10,19 ab) was not significantly inhibited compared to the untreated control plants (11,09 a).

The additional application of plant biostimulant has an improving effect received 20% Derby Super, where the physiological parameters do not differ statistically from that in control plants on herbicide treated oilseed rape plants. This ameliorative effect is more pronounced on the plants. Our results correspond with to those reported by Balabanova et al. (2016), where have been found similar ameliorative effect of plant biostimulant containing amino acids on sunflower photosynthetic apparatus after herbicide inhibition.

The number of siliques plant⁻¹ is decisive for the seed yield (Diepenbrock, 2000). The results regarding the silique number per plant is presented on Table 2.

The highest silique number per plant for the untreated control and variant 5 (Derby Super -6.6 g ha⁻¹ + Amino Expert Impuls -3.0 l ha⁻¹) was recorded. The silique number of these two treatments was 219.73 and 203.13 average for the period respectively. The results showed the medicative effect of the biostimulant application which is more pronounced at the rate of Derby Super -6.6 g. and lower for the 100% rate of the studied herbicide product.

The absolute seed mass is a very important quality indicator. The results from our study are presented in Table 3. According to Georgiev et al. (2014) this indicator is crucial for the formation of the yields. The highest absolute seed mass for the untreated control and for treatment 5 (Derby Super + Amino Expert Impuls) was recorded – 4.37 and 4.32 g average for the period respectively.

At treatment 4 the absolute seed mass is 4.17 g. The lowest results are found to be for the treatments without medicative application of biostimulant. The obtained data showed that this quality indicator could be improved with biostimulant application in case of herbicide drift of Derby Super on the field.

Treatments	Ch a, mg/g FW	Ch b, mg/g FW	Carotenoids, mg/g	Net photosynthetic
			FW	rate, mmol CO ₂ m ⁻² s ⁻¹
1. Untreated control	1.572 a	0.145 a	0.135 a	11.09 a
2. Derby Super 100%	1.288 c	0.074 c	0.051 c	8.53 b
3. Derby Super 20%	1.415 b	0.130 ab	0.122 a	10.19 ab
4. Derby Super 100% + Amino Expert Impuls	1.459 ab	0.110 b	0.095 b	9.63 ab
5. Derby Super 20% + Amino Expert Impuls	1.475 ab	0.131 ab	0.112 ab	10.92 a

Values with different letters are with proved differences according to Duncan's Multiple Range test (p < 0.05)

Treatments	2018	2019	2020	Average
1. Untreated control	190.50 a	229.90 a	238.80 a	219.73 a
2. Derby Super 100%	143.10 c	164.40 d	186.30 c	164.60 d
3. Derby Super 20%	170.60 b	190.20 c	203.60 b	188.13 ab
4. Derby Super 100% + Amino Expert Impuls	157.00 b	187.40 c	193.27 b	179.22 c
5. Derby Super 20% + Amino Expert Impuls	184.80 a	206.00 b	218.60 a	203.13 ab

Table 2. Silique number plant⁻¹

Values with different letters are with proved differences according to Duncan's Multiple Range test ($p \le 0.05$)

Table 3. Absolute seed mass of 1000 seeds, g

Treatments	2018	2019	2020	Average
1. Untreated control	4.16 a	4.34 a	4.61 a	4.37 a
2. Derby Super 100%	3.32 c	3.55 b	3.81 c	3.56 c
3. Derby Super 20%	3.81 b	3.68 b	4.06 bc	3.85 bc
4. Derby Super 100% + Amino Expert Impuls	3.90 b	4.22 a	4.39 a	4.17 ab
5. Derby Super 20% + Amino Expert Impuls	4.15 a	4.29 a	4.51 a	4.32 a

Values with different letters are with proved differences according to Duncan's Multiple Range test (p < 0.05)

In a study conducted by Vujaković et al. (2014) the hectoliter seed mass of oilseed rape depended on genotype, planting density and the year. In our study the results during the different experimental seasons were also different. The highest hectoliter mass of the oilseed rape seeds was recorded for variants 1 and 4 and 5 - 66.85, 66.15 and 66.32 kg respectively (Table 4). As well as for the indicator absolute seed mass, for this parameter, a tendency of improving after the medicative application of biostimulant independently the rate of Derby Super was recorded.

The damage caused by the herbicide drift can lead to severe yield losses (Schroeder et al., 1983; Dexter, 1993; Roider et al., 2008; Constantin et al., 2016). Some studies support the statement that the application of biostimulants can improve plant biomass, yield and resistance to multiple types of stress (Calvo et al., 2014; Constantin et al., 2016; Nardi et al., 2016; Panfili et al., 2019). The obtained data from our research corresponds with the findings of the authors cited above (Table 5). The highest oilseed rape seed yield for the untreated control was recorded -3.35 t ha⁻¹ average for the period. The results found for treatment 5 where Derby Super -6.6 g ha⁻¹ + Amino Expert Impuls -3.0 l ha⁻¹ was applied were 3.05 t ha⁻¹. The obtained results are not significantly different whit those of the untreated control according to Duncan's Multiple Range test (p < 0.05).

The medicaive application of Amino Expert Impuls for the treatment with Derby Super in its registered rate (variant 4) could not improve the yield performance of the oilseed rape and had approximately 50% lower yield according to the untreated control and variant 5. The lowest seed yields for variant 2 were fond -1.45 t ha⁻¹.

Table 4. Hectoliter seed mass, kg

Treatments	2018	2019	2020	Average
1. Untreated control	66.32 a	67.28 a	66.96 a	66.85 a
2. Derby Super 100%	62.82 d	63.42 c	62.54 c	62.93 c
3. Derby Super 20%	64.66 c	65.08 b	65.27 b	65.00 b
4. Derby Super 100% + Amino Expert Impuls	65.98 b	66.08 a	66.40 a	66.15 a
5. Derby Super 20% + Amino Expert Impuls	66.60 a	66.99 a	65.38 b	66.32 a

Values with different letters are with proved differences according to Duncan's Multiple Range test (p < 0.05)

Table 5. Oilseed rape seed yields, t ha-1

Treatments	2018	2019	2020	Average
1. Untreated control	3.42 a	3.61 a	3.02 a	3.35 a
2. Derby Super 100%	1.23 d	1.68 c	1.46 c	1.45 c
3. Derby Super 20%	1.96 c	2.59 b	2.67 b	2.41 b
4. Derby Super 100% + Amino Expert Impuls	1.25 d	2.09 bc	1.81 c	1.72 c
5. Derby Super 20% + Amino Expert Impuls	2.95 b	3.14 b	3.07 a	3.05 a

Values with different letters are with proved differences according to Duncan's Multiple Range test ($p \le 0.05$)

Conclusions

The herbicide drift caused phytotoxic effect on oilseed rape which was stronger for the high and registered rate of the studied herbicide product (Derby Super).

The plants received additional therapeutic application by biostimulant recovered faster and formed higher silique number per plant as well as biological yield.

The increased rates of the studied physiological parameters confirmed the ameliorative effect of the plant biostimulant application.

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