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HERBICIDE STRESS AND BIOSTIMULANT APPLICATION INFLUENCES THE LEAF N, P AND K CONTENT OF SUNFLOWER

Nesho NESHEV

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: n_neshev85@abv.bg

Abstract

The aim of the study conducted between 2017 and 2019 is to evaluate the influence of the herbicide stress caused by imitation of "mistaken" treatment of the ExpressSun® sunflower hybrid P 64 LE 25 with imazamox to the leaf N, P and K content of plants before flowering stage. Treatments of the experiment were: 1. Untreated control; 2. Pulsar® Plus - 2.00 l ha^{-1} (25 g/l imazamox); 3. Pulsar® Plus - 2.00 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1} (biostimulant). The herbicide was applied in 4th - 6th true leaf stage of the crop. At treatment 3, biostimulant was applied four days after the herbicide intervention. An increase in the leaf N content after the medicative biostimulant application average for the period was found. No influence of the "mistaken" treatment on the P content in the leaves was recorded. The plants treated "mistakenly" with Pulsar® Plus (2.00 l ha^{-1}) had increased K levels in the leaves. This increased K content was probably due to high abiotic stress. K, in turn, helps the plants to overcome the stressful conditions to some extent.

Key words: *herbicide stress, biostimulants, sunflower, NPK in leaves.*

INTRODUCTION

Bulgaria and Romania are the largest sunflower producers in the EU (Hristov et al., 2019). In order to achieve high yields is necessary to control the weeds effectively in the optimal phases of the crop before the critical period for decreasing the morphological parameters is reached (Tonev, 2000; Simic et al., 2011). In agriculture, the weed control is based mainly on chemical control. A great number of researchers are working on the chemical weed control in sunflower and other crops (Goranovska and Yanev, 2016; Kostadinova et al., 2016; Mitkov et al., 2016; Tityanov, 2016;Yanev, 2015; Tonev et al., 2010a; Tonev et al., 2010b; Tonev et al., 2009a; Tonev et al., 2009b). The main reason for the considerable success of modern herbicides is their selective action (Cobb and Reade, 2010). A selective herbicide is one that kills or retards the growth of weeds, while causing little or no injury to crop species (Carvalho et al., 2009). Two types of mechanisms conferring resistance to herbicides in weeds and crops are currently described. The mechanisms that are related to the specific site of action of the herbicide in the plants are known as target site mechanisms, while the others that involve processes not related to the mechanism by which herbicides kill plants are known as non-target site mechanisms (Hanson et al., 2007). Independently that the modern herbicides are considered selective for the crop plants, in some conditions they can be toxic for the crops and can cause herbicide phytotoxicity (Tonev and Vassilev, 2011). Herbicides have distinct target sites where they act to disrupt biochemical process leading to cell, tissue or plant death. The majority of target sites are specific enzymes that play a key role in a plant metabolic process, which dysfunction has lethal consequences to the susceptible plant. Herbicide selectivity could be derived also from target-enzyme insensitivity “site of action resistance” (Devine et al., 1993).

Herbicide phytotoxicity can occur after long-term effects and herbicidal drift on non-target crops (Vischetti et al., 2002). This undesirable phenomenon can be observed in the low-volume spray with droplet size lower than 150 μm (Hanks, 1995), as well as in conditions of inappropriate wind speed and direction (Thistle, 2004). Herbicides can have a toxic effect on crops in several major cases: 1. In case of technological mistakes - increase in the herbicide dose; 2. Due to insufficient selectivity; 3. By application of the herbicides in unsuitable weather conditions (drought, waterlogging, low temperatures, etc.) as well as in the driftage of the herbicide on another crop (herbicide drift); 4. Application of herbicide by mistake on crop that is sensitive; etc.

The tolerant plants have the ability to detoxify the herbicide, with this process occurring in 3 phases (Yuan et al., 2007; Cole, 1994). In the first phase, the herbicide molecules that enter the cell undergo oxidation or hydrolysis, providing functional groups that are suitable for subsequent conjugation with endogenous metabolites (Barrett et al., 1995). In the second phase, once oxidized herbicides are conjugated with glucose or the metabolite glutathione, these reactions are catalyzed by enzymes including glycosyl transferases and/or glutathione S-transferases (Lamoureux et al, 1991). The third phase of xenobiotic metabolism is the transport and storage of herbicide conjugates in the cellular vacuole (Yuan et al., 2007).

When herbicide detoxification is not effective enough, various functional impairments occur in plants. Herbicide phytotoxicity is most often chronic, but in some cases (eg. drift) it can also be fatal to the crop. The extent of damage can be assessed visually (if visible) or by various physiological and biochemical indicators (Dayan et al., 2015; Dayan and Zaccaro, 2012).

Ability to recover the herbicide-damaged plants depends on the degree of the occurred structural-functional impairment. A number of studies have shown that chronic herbicide phytotoxicity can be overcome (to some extent or completely) by application of biostimulants, foliar fertilizers, growth regulators, herbicide antidotes, etc. (Jablonkai, 2013).

Tanev (1987) demonstrated that the low molecular weight fractions of humic substances extracted from manure exhibit herbicide phytotoxicity antidote properties. Balabanova et al. (2016) show that the biostimulant Terra-Sorb Foliar significantly restores the physiological status of IMI-R sunflower plants treated with the herbicide product Pulsar 40 (imazamox). Soltani (2015) reported a significant increase in yields of oats and winter wheat as a result of the addition of biostimulant after vegetative treatment of the crops with the herbicides glyphosate, topramezone and atrazine.

There is limited information for the influence of the herbicide stress on the nutrient status of the crops. Zaidi et al. (2005) found that nitrogen content in plants decreased with increasing herbicide dose.

The aim of the research is to evaluate the influence of the herbicide stress after imitation of mistaken treatment of ExpressSun® sunflower hybrid with imazamox (Pulsar® Plus) and the medicative biostimulant application on the leaf N, P and K content of sunflower before flowering stage.

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 is 28 m². The study included the following treatments: 1. Untreated weed free control; 2. Pulsar® Plus 2.00 l ha^{-1}; 3. Pulsar Plus® 2.00 l ha^{-1} + Amino Expert® Impuls 2.00 l ha^{-1}.

Pulsar® Plus (25 g/l imazamox) is herbicide for weed control in the Clearfield® Plus Technology of sunflower. The biostimulant's content is as follows: Amino acids - 58.00 g/l; N - 29.37 g/l; MgO - 5.93 g/l; SO_3 - 46.66 g/l, Phytohormones - 0.0035 g/l; Micronutrients: B - 6100 mg/l; Cu - 4600 mg/l; Fe - 4520 mg/l; Mn - 4520 mg/l; Mo - 920 mg/l; Zn - 9040 mg/l. The herbicide was applied in 4^{th} – 6^{th} true leaf stage of the sunflower (BBCH 14-16). The size of the spraying solution was 220 l ha^{-1}. The biostimulant was applied 4 days after the herbicide intervention. The grown sunflower hybrid was P 64 LE 25 - bred to be grown by the Express Sun® Technology of sunflower.

A predecessor of the sunflower in the three years of the study was winter wheat. On the trial field deep ploughing, two times disc harrowing and two times cultivation before sowing were done. The application was performed after sowing before germination of the crop. On the whole experimental area basic combine fertilization with 250 kg ha^{-1} NPK (15:15:15) and spring dressing with 200 kg ha^{-1} NH_4NO_3 was performed.

To determine the content of nutrient elements in the leaves before flowering stage of the sunflower the last fully developed leaves of the crop were collected. The plant samples were dried at 60°C, weighted and milled. They were mineralized with concentrated H_2SO_4 using H_2O_2 as a catalyst. The total nitrogen content was determined according to Kjeldahl method by distillation in apparatus of Parnas-Wagner (Tomov et al., 2009). Phosphorus was determined colorimetrically (spectrophotometer Camspec E105) (Tomov et al., 2009) and potassium - photometrically (flame photometer PFP-7) (Ivanov and Krastev, 2005).

The herbicide phytotoxicity was determined by the 9-score scale of EWRS (European Weed Research Society) 7 days after the herbicide application as followed:

1. No damage/healthy plant;
2. Very slight symptoms, weak suppression;
3. Slight but clearly visible symptoms;
4. Severe symptoms (e.g. chlorosis) which do not lead to a negative effect on yield;
5. Thinning, severe chlorosis or suppression; yield reduction expected;
6. Severe damage up to complete destruction;
7. Severe damage up to complete destruction;
8. Severe damage up to complete destruction;
9. Severe damage up to complete destruction.

Statistical analysis of collected data was performed by using Duncan's multiple range test by the software SPSS 19. Statistical differences were considered proved at $p < 0.05$.

RESULTS AND DISCUSSIONS

The ecological conditions at the time of the herbicide application and the first days after that had the strongest influence on the retention and adsorption of the foliar herbicides, as well as the subsequent processes that determine their action (Tonev et al., 2007).

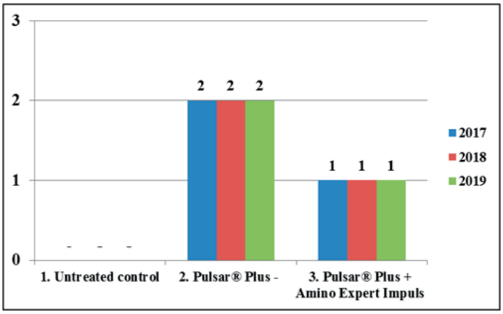

Figure 1. Visual phytotoxicity 7 days after the treatments (Scores)

Sulphonylureas (tribenuron-methyl) and imidazolinones (imazamox) are herbicides inhibiting ALS (Acetolactate synthase), an enzyme that plays an important role of the biosynthesis of the vital amino acids valine, leucine and isoleucine. The visual toxic symptoms in the sensitive or non-target plants are growth retardation or stopping, the leaves lose their turgor, the plants lose their vitality, etc. (Fedke and Duke, 2005).

The visual phytotoxicity at treatment 2 (Pulsar® Plus 2.00 l ha^{-1}) was determined as score 2. Symptoms of yellowing of the plant's leaves were observed. At treatment 3, after the medicative biostimulant application, the phytotoxic symptoms were determined as score 1 and the plants were healthy.

The optimal N levels in the upper fully developed leaves in the beginning of flowering stage (BBCH 61) vary from 3.00 to 5.00% (Bergmann, 1992). After the "mistaken" treatment with Pulsar® Plus at the studied hybrid P 64 LE 25, the nitrogen content in the leaves in the beginning of flowering was diminished in comparison to the untreated weed free control. Increase in the N content in this phenophase after the medicative application of the biostimulant Amino Expert® Impuls was found. This is probably due to the biostimulant that contents aminoacids, macro- and micronutrients. Coupled with the higher tolerance of the hybrid to herbicide stress is probably the reason for the increase of N in leaves. The results are presented in Table 1.

Table 1. Nitrogen content in the leaves before flowering of the sunflower (%)

Treatments	2017	2018	2019	Ave.
1. Untreated control	4.10b	4.44a	5.28a	4.61 a
2. Pulsar® Plus - 2.00 l ha^{-1}	3.88c	3.97b	4.65b	4.17 b
3. Pulsar® Plus - 2.00 l ha^{-1} + Amino Expert® Impuls - 2.00 l ha^{-1}	4.51a	4.82 a	5.12a	4.82 a

All values with different letters are with proved difference according to Duncan's test, $p < 0.05$.

The optimal phosphorus levels in the upper fully developed leaves in the beginning of flowering stage (BBCH 61) vary from 0.25 to 0.50% (Bergmann, 1992).

In the trial the plants had higher phosphorus content in the leaves (Table 2). It was found that the plants treated “by mistake” with imazamox showed higher phosphorus content in the leaves before flowering stage - 0.92% average for the period of investigation. The difference in this result is with proved difference according to Duncan’s test, $p < 0.05$ when compared to the untreated control and the variant with applied medicative biostimulant application (treatment 3).

Table 2. Phosphorus content in the leaves before flowering of the sunflower (%)

Treatments	2017	2018	2019	Ave.
1. Untreated control	0.83b	0.88b	0.89b	0.87b
2. Pulsar® Plus - 2.00 l ha^{-1}	0.91a	0.95a	0.90a	0.92a
3. Pulsar® Plus - 2.00 l ha^{-1} + Amino Expert® Impuls - 2.00 l ha^{-1}	0.81b	0.86b	0.89b	0.85b

All values with different letters are with proved difference according to Duncan’s test, $p < 0.05$.

The optimal potassium levels in the upper fully developed leaves in the beginning of flowering stage (BBCH 61) vary from 3.00 to 4.50% (Bergmann, 1992).

Table 3. Potassium content in the leaves before flowering of the sunflower (%)

Treatments	2017	2018	2019	Ave.
1. Untreated control	3.57b	3.42b	3.89a	3.63b
2. Pulsar® Plus - 2.00 l ha^{-1}	3.83a	4.26a	3.99a	4.03a
3. Pulsar® Plus - 2.00 l ha^{-1} + Amino Expert® Impuls - 2.00 l ha^{-1}	3.02c	3.16c	3.30b	3.16c

All values with different letters are with proved difference according to Duncan’s test, $p < 0.05$.

In the experiment a treatment with lower phosphorus levels under the optimal values was not found. The obtained data is presented in Table 3. Despite the optimal content of potassium in the leaves, it was found that the plants treated "mistakenly" with Pulsar® Plus - 2.00 l ha^{-1} had increased potassium levels in the leaves before flowering - 4.03%, which is with 0.40% higher than the untreated control - 3.63% on average for the period of the study. This increased content is probably due to the fact that the plants of treatment 2 are subjected to high abiotic stress, and potassium in turn helps the plants to overcome the stress conditions to some extent (Nikolova, 2010). It seems the probable reason for the higher potassium content of the leaves of the plants from treatment is the caused herbicide stress.

CONCLUSIONS

After the application of the biostimulant Amino Expert® Impuls for medicative treatment, the plants of treatment 2 overcome the herbicide stress to some extend;

The plants of all variants had optimum nitrogen content in the leaves before flowering, but stressed by "wrong" treatment with Pulsar® Plus had a reduced nitrogen content in the leaves before flowering;

The plants treated “by mistake” with imazamox had higher phosphorus content in the leaves before flowering stage;

Despite the optimum content of potassium in the leaves, plants mistakenly treated with imazamox had increased potassium levels in the leaves before flowering. The probable reason for the higher potassium content of the leaves of the plants is the caused abiotic stress

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