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THE N, P AND K CONTENT IN PUMPKIN'S LEAVES IS INFLUENCED BY HERBICIDE STRESS AND BIOSTIMULANT APPLICATION

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Abstract

The aim of the study conducted from 2017 to 2019 is to evaluate the influence of the herbicide stress caused by the herbicide imazamox and biostimulatory treatment (preventive and curative) to the leaf N, P, and K content of pumpkins before the flowering stage. In the trial, the pumpkin variety 'Mathilda' F1 was grown. The experiment included 12 treatments. Number 1 was untreated weed-free control. Treatment 2 was Pulsar® 40 (40 g/l imazamox) applied at a rate of 1.00 l ha^{-1}. The other treatments (from 3 to 7) represented the application of the mentioned herbicide in tank mixture with biostimulant. The treatments from 8 to 12 showed the performance of therapeutic biostimulant application 7 days after the herbicide spraying. The herbicide was applied in BBCH 12-13 of pumpkins. The biostimulant products evaluated were: Shigeki®; Amino Expert® Impuls; Lactofol® O; Aminozol®; Terra-Sorb® Complex. The highest leaf N content for the untreated control was found. No influence of the treatments on the P content in the leaves was observed. The treatments that received the highest herbicide stress had increased leaf K levels.

Key words: *herbicide stress, biostimulants, pumpkins, NPK in leaves*

INTRODUCTION

Presence of weed infestation is one of the most limiting factors leading to rapid yield decrease. There is a large number of authors working on the weed control in the different crops (Yanev, 2020; Manilov and Zhalnov, 2018; Goranovska and Yanev, 2016; Kostadinova et al., 2016; Mitkov et al., 2016; Tityanov et al., 2016; Yanev, 2015; Yanev et al., 2014a; Týr and Vereš, 2012; Tonev et al., 2010a; Tonev et al., 2010b; Tonev et al., 2009a; Tonev et al., 2009b; Changsaluk et al., 2007; Masqood et al., 1999; Plew et al., 1994). In the fields of the late spring crops as pumpkins mainly late-spring weeds are developing.

The most distributed broadleaf weed species are common amaranth (*Amaranthus retroflehus* L.), wild mustard (*Sinapis arvensis* L.), fat-hen (*Chenopodium album* L.), wild hemp (*Cannabis ruderalis* L.), common cocklebur (*Xanthium strumarium* L.), creeping thistle (*Cirsium arvense* L.), etc.

The most distributed grass weed species are yellow foxtail (*Setaria* spp.), barnyard grass (*Echinochloa crus-galli* L.), johnson grass (*Sorghum halepense* L. (Pers.)) (Tonev et al., 2007).

The chemical control is one of the most commonly used weed control methods in crops. The proper herbicide choice is one of the most important and responsible parts of crop management. The proper herbicide must meet a number of requirements. It should be selective for the crop, highly effective against the weeds, its application rates should not lead to the accumulation of residues in plant production and in soil, it should not deteriorate the quality of production and it should be harmless to microorganisms in soil, as well as for the environment (Yanev et al., 2014b; Hristeva et al., 2014; Hristeva et al., 2015; Kalinova and Yanev, 2015; Semerdjieva et al., 2015; Yanev and Kalinova, 2020). Pumpkins are sensitive to herbicides and the chemical weed control is quite limited at that crop (Tonev et al., 2007).

When herbicide detoxification is not effective enough, various functional impairments may occur. A selective herbicide destroys or retards the growth of weeds, while causing little or no injury to crop species (Carvalho et al., 2009).

Herbicide phytotoxicity is most often chronic, but in some cases it can parish 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).

There are a great number of registered herbicides for grass weed control in pumpkins, but almost none for broadleaf weeds elimination.

The herbicide imazamox (that controls a wide spectrum of broadleaf as well as grass weeds) can be applied in pumpkins, but the application of the product Pulsar 40 (40 g/l imazamox) in rate of 1.00 l ha^{-1} caused visual phytotoxic symptoms to the pumpkins in the study. Also, the application of the herbicide imazamox with the biostimulant Amino Expert Impuls in tank mixture showed a protective effect to the plants (Neshev et al., 2020).

There is limited information for the effect of the herbicide stress on the plant's nutrient status. Zaidi et al. (2005) found that plant's N content lowered with increasing the herbicide dose.

The aim of the current research is to evaluate the influence of the herbicide stress caused by the herbicide imazamox and biostimulant treatment (preventive and medicative) to the leaf N, P, and K content of pumpkins before flowering stage.

MATERIALS AND METHODS

The experiment was situated in the experimental field of the Agricultural University of Plovdiv, Bulgaria. The trial was conducted by the randomized block design in 3 replications.

Studied products and evaluations:

Herbicide product: Pulsar® 40 (40 g/l imazamox). It is registered for Clearfield Technology at sunflower. This technology offers the farmers new and effective solution for weed control (Pfenning et al., 2008). Imazamox is applied as a spray when the sunflower plants are at 4-10 leaves stage (Kamburoglu et al., 2019).

Products with biostimulant mode of action:

- Shigeki® - Extract of algae (*Ascophyllum nodosum*) - 15%; Macronutrients: P_2O_5 - 7%; K_2O - 10%; Microelements: Fe-EDTA - 0.25%; Mn-EDTA - 0.17%; Zn-EDTA - 0.20%; Cu-EDTA - 0.10%; B - 0.20%; Mo - 0.04%.
- 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%.
- Lactofol® O (Macroelements: N - 21% = NO_3 - 7%, NH_4 - 4% and amide - 10%); P_2O_5 –-5%; K_2O - 10%; SO_3 - 0.6%; Microelements: B - 0.02%; Cu - 0.014%; Fe - 0.025%; Mn - 0.018%; Mo - 0.002%; Zn - 0.01%.
- Aminozol® - Macroelements: N - 9.4%; K_2O - 1.1; S - 0.25%; Na - 1.28 %; 66.3% organic substances obtained from animal sub products 3rd category EG (VO) 1069/2009; protein hydrolysates.
- Terra-Sorb® Complex - Macroelemets: N - 5.5%; MgO - 0.8%; Microelements: B - 1.5%; Fe - 1.0%; Mn - 0.1%; Zn - 0.1%: Mo - 0.001%; Free amino acids - 20%.

The study included the following treatments:

1. Untreated weed free control;
2. Pulsar® 40 1.00 l ha^{-1};
3. Pulsar® 40 1.00 l ha^{-1} + Shigeki® 3.00 l ha^{-1};
4. Pulsar® 40 1.00 l ha^{-1} + Amino Expert® Impuls 3.00 l ha^{-1};
5. Pulsar® 40 1.00 l ha^{-1} + Lactofol® O 6.00 l ha^{-1};
6. Pulsar® 40 1.00 l ha^{-1} + Aminozol® 3.00 l ha^{-1};
7. Pulsar® 40 100 ml da^{-1} + Terra-Sorb® Complex 3.00 l ha^{-1};
8. Pulsar® 40 1.00 l ha^{-1} + Shigeki® 3.00 l ha^{-1};
9. Pulsar® 40 1.00 l ha^{-1} + Amino Expert® Impuls 3.00 l ha^{-1};
10. Pulsar® 40 1.00 l ha^{-1} + Lactofol® O 6.00 l ha^{-1};
11. Pulsar® 40 1.00 l ha^{-1} + Aminozol® 3.00 l ha^{-1};
12. Pulsar® 40 1.00 l ha^{-1} + Terra-Sorb® Complex 3.00 l ha^{-1}.

Variant 2 was treated with imazamox only in BBCH 12-13 and did not receive preventive or curative biostimulant application.

At variants from 3 to 7 the treatment was accomplished in BBCH 12-13, but the plants were sprayed with tank mixture of the herbicide and the different biostimulants – preventive approach.

At variants from 8 to 12, firstly the application of Pulsar 40 was done in BBCH 12-13, and 7 days later (in BBCH 19-21), treatment with the different biostimuants was performed – therapeutic approach. The size of the spraying solution was 500 l ha^{-1} for all treatments.

Predecessor of pumpkins in each year was maize. The performed tillage operations after predecessor's harvesting were deep ploughing and two times harrowing before planting. The crop was planted as preliminary grown seedlings with planting distance 1 x 1.5 m (6670 plants ha^{-1}).

For control of annual and perennial grass weeds 3-5 leaf stage of the annual weed species and 10-20 cm of height of Johnson grass (*Sorghum halepense* (L.) Pers.) application with Stratos® Ultra (100 g/l cycloxydim) in rate of 2.00 l ha^{-1} was done.

The whole experimental area was fertilized with 250 kg ha^{-1} NPK 15:15:15 after predecessor's harvest and tillage operations as well as 250 kg ha^{-1} NH_4NO_3 in spring before planting of the pumpkins.

To determine the content of nutrient elements in the leaves before flowering stage of the pumpkins 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 Parnas - Wagner apparatus (Tomov et al., 2009). Phosphorus was determined colorimetrically on spectrophotometer Camspec E105 (Tomov et al., 2009) and potassium - photometrically by flame photometer PFP-7 (Ivanov and Krastev, 2005).

The visual herbicide phytotoxicity was determined by the 9-score scale of EWRS (European Weed Research Society) on the 7th day 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

Despite the visual phytotoxic symptoms caused by the herbicide application for all treatments (with or without biostimulant application) the herbicide phytotoxicity on the 7th day after treatment varied from score 1 to score 3 in the different years of the research. The highest herbicide stress was found for variant 2 (Pulasr 40 alone), as well as for the treatments of Lactofol® O in tank mixture with imazamox or 7 days after the herbicide application – variants 5 and 10 respectively. The foliar fertilizer Lactofol® O contains high percent of nitrogen in its formulation (21%) that may be the cause of the high phytotoxic effect. In research findings it is reported that the co-application of fertilizers with different herbicides can cause injuries to the crop (Soltani et al, 2012).

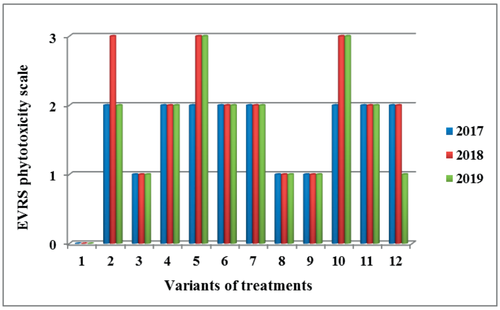

Figure 1. Visual phytotoxicity 7 days after the herbicide application (scores)

Chen et al. (1998) set optimum concentrations for nitrogen, phosphorus and potassium from fertilizer experiments conducted in Guangdong, with the leaves collected in autumn before flower initiation.

The leaf nitrogen (N) levels in the beginning of pumpkin's flowering are presented on Table 1.

Table 1. Nitrogen content in the pumpkin's leaves in the beginning of flowering stage (BBCH 61), %

Tr.	2017	2018	2019	Average
1	3,24 a	3,36 a	3,88 a	3,49 a
2	2,25 e	1,92 e	2,06 d	2,04 d
3	2,73 cd	2,63 cd	2,99 c	2,78 c
4	2,69 cd	2,55 d	2,90 c	2,71 c
5	2,32 e	2,16 e	2,00 e	2,16 d
6	2,58 d	2,62 cd	2,99 c	2,73 c
7	2,84 c	2,72 c	3,16 c	2,91 bc
8	2,66 d	2,51 d	2,97 c	2,71 c
9	2,77 c	2,64 cd	2,84 c	2,75 c
10	2,23 e	2,10 e	1,59 f	1,97 d
11	3,17 b	3,00 b	3,64 b	3,27 ab
12	2,70 cd	2,81 c	3,10 c	2,87 bc

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

The optimal N content in the leaves of pumpkins vary from 3.00 to 6.00 % (Hochmuth et al., 2004). With the highest and optimal nitrogen content in the leaves according to these authors was variant 1 (Untreated weed free control) – 3.49% average for the period. The N content for this variant was with proved difference with the other treatments according to Duncan's multiple range test ($p < 0.05$). With 3.27% N in the leaves were the plants of treatment 11 (Pulsar® 40 1.00 l ha^{-1} in BBCH 12-13 + Aminozol® 3.00 l ha^{-1} in BBCH 19-21).

At all treatments, the foliar N concentration is below 3.00% and is under the optimal nutritional levels. Average for the period of the study, the lowest N content in the leaves of treatments 2, 5 and 10 was recorded – 2.04, 2.16 and 1.97% respectively. Most likely, herbicidal stress leads to disturbances in nitrogen metabolism in pumpkins and plants experience nitrogen deficiency, which later may have a negative impact on plant development.

The optimal phosphorus content in pumpkin leaves is from 0.30 to 0.50% (Silva and Uchida, 2000; Hochmuth et al., 2004).

The plants of variant 5 (Pulsar® 40 + Lactofol® O in a tank mixture) had the lowest phosphorus content - 0.52% on average for the experimental period.

The variants 2 (Pulsar® 40 1.00 l ha^{-1}) and 10 (Pulsar® 40 1.00 l ha^{-1} in BBCH 12-13 + Lactofol® O in BBCH 19-21) had a content of 0.54 and 0.56% phosphorus in the leaves before flowering of the plants, respectively.

Table 2. Phosphorus content in the pumpkin's leaves in the beginning of flowering stage (BBCH 61), %

Tr.	2017	2018	2019	Average
1	0,61 b	0,60 b	0,62 a	0,61 b
2	0,52 c	0,55 c	0,54 b	0,54 cd
3	0,60 b	0,63 ab	0,63 a	0,62 b
4	0,62 b	0,61 b	0,63 a	0,62 b
5	0,55 c	0,50 c	0,51 b	0,52 d
6	0,62 b	0,59 b	0,61 a	0,61 b
7	0,60 b	0,59 b	0,63 a	0,61 b
8	0,60 b	0,60 b	0,64 a	0,61 b
9	0,59 b	0,58 bc	0,52 b	0,56 c
10	0,53 c	0,55 c	0,59 ab	0,56 c
11	0,69 a	0,65 a	0,68 a	0,67 a
12	0,62 b	0,63 ab	0,66 a	0,64 ab

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

The pumpkin plants from variant 11 (Pulsar® 40 1.00 l ha^{-1} in BBCH 12-13 + Aminozol® 3.00 l ha^{-1} in BBCH 19-21) had the highest phosphorus content (0.67%) on average for the experimental period.

The optimal potassium content in pumpkin leaves is from 2.30 to 4,00% according to Hochmuth et al. (2004) and from 3.00 to 5,00% according to Silva and Uchida (2000). The maintenance of optimum K nutrition status is very important for plant resistance to biotic and abiotic stresses and from other hand this nutrient element is helping the plants to overcome the stress conditions (Wang et al. (2013; Nikolova M., 2010). The results for the content of potassium in the leaves of pumpkin plants at the beginning of flowering are presented on Table 3.

Table 3. Potassium content in the pumpkin's leaves in the beginning of flowering stage (BBCH 61), %

Tr.	2017	2018	2019	Average
1	3,25 e	3,24 e	3,67 d	3,39 b
2	4,07 b	4,28 b	4,88 a	4,41 a
3	3,49 d	3,29 e	3,12 e	3,30 b
4	3,65 c	3,47 d	4,13 b	3,75 b
5	4,32 a	4,51 a	4,99 a	4,61 a
6	3,55 d	3,31 e	3,81 bc	3,56 b
7	3,29 e	3,44 d	3,94 b	3,56 b
8	3,59 c	3,66 c	3,80 bc	3,68 b
9	3,50 d	3,40 d	3,95 b	3,62 b
10	4,21 a	4,42 ab	4,80 a	4,48 a
11	3,46 d	3,65 c	4,01 b	3,71 b
12	3,14 e	3,33 e	3,54 d	3,34 b

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

On average for the study period, all plants had an optimal content of potassium in the leaves before flowering stage. In stressed plants from variants 2, 5 and 10 an increase in potassium levels in leaves was found - 4.41, 4.61 and 4.48%, respectively. Probably the plants of these variants absorb and accumulate higher quantities of this macronutrient in their leaves. This increased content is probably due to the high abiotic stress caused by the herbicide application and the inappropriate chose of foliar fertilizer for preventive and therapeutic treatment of the stressed plants, and potassium is the element helping the plants to overcome the conditions of stress (Nikolova M., 2010).

CONCLUSIONS

The application of the herbicide Pulsar 40 (40 g/l imazamox) in a rate of 1.00 l ha^{-1} caused temporary phytotoxic symptoms to the pumpkins on the 7^{th} day after treatments. In time, the plants overcome the herbicide toxicity to some extent.

The highest N content in the leaves before flowering were recorded for the untreated control.

The highest P levels for the plants of the treatment with Pulsar® 40 1.00 l ha^{-1} in BBCH 12-13 + Aminozol® 3.00 l ha^{-1} in BBCH 19-21 were measured.

Increasing levels of K for the treatments with highest symptoms of phytotoxicity were recorded.

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