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How photosynthetic light phase of spiny cocklebur (Xanthium spinosum L.) changes during times after herbicide application?

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Abstract
The photosynthetic light phase of spiny cocklebur (*Xanthium spinosum* L.) plant was studied by JIP-test at different times (12, 36, 60 and 84 hours) after the application of nicosulfuron (®Cruz) and bentazon (®Basagran) herbicides. Results indicated that the application of nicosulfuron did not affect most of the chlorophyll *a* fluorescence (ChlF) parameters. But, the application of bentazon significantly decreased PSII activity due to increasing F0 and decreasing Fm, Fv, Fv/F0 and especially Fv/Fm. The maximum effect of bentazon was observed on the donor side of PSII by increasing ABS/RC and decreasing TRo/RC and also by reduction of φ_{E0}. Reduction in photosynthesis relative vitality (PI) of photosynthesis apparatus by bentazon affected photosystem II (PSII) reaction centers, Fv/Fm and φ_{E0}. The highest negative effect of bentazon on PSII activity was recorded at 36 hours after herbicide application. Bentazon via increasing K_P, decreased electron transportation.

Keywords: Bentazon; Chlorophyll *a* fluorescence; Electron transport; JIP-test; Nicosulfuron; Spiny cocklebur.

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Introduction

Among all herbicides which are used in crop protection, nearly 50% affect the chloroplast. Several herbicides such as benzothiadiazinones are known as inhibitors of photosystem II (PSII) activity (Cobb and Read 2010). Bentazon (Basagran, SL48%) which belongs to the benzothiadiazinones is a very strong inhibitor (Grumbach 1982). This herbicide is used for the control of broadleaf weeds in crops such as corn and soybean (Cobb and Read 2010). Apart from inhibiting electron transport, many herbicides have more than one side of action. Photosystem II inhibitor herbicides like bentazon also alter chloroplast ultrastructure and pigment composition (Grumbach 1982). Nicosulfuron (Cruz, SC4%) is a sulfonylurea herbicide being considered for registration for post-emergence weed control in crops such as corn (Williams and Gilham 1990). Sulfonylureas are a class of herbicides that inhibit the activity of acetohydroxyacid synthase/acetolactate synthase and decrease the synthesis of valine, leucine and isoleucine as the branched- chain amino acid (Ray 1984; Cobb and Read 2010).

Many stresses such as herbicide application on plants can affect the photosynthetic activity of leaves and change the ChlF parameters (Oukarroum *et al.* 2007; Kalaji and Guo 2008; Hassannejad *et al.* 2020). ChlF analysis as a rapid, non-intrusive and inexpensive method, provides detailed information on the energy flow in the photosynthetic apparatus (Oukarroum *et al.* 2007). This method can be used for understanding the stressful effects of herbicides on weeds (Hassannejad *et al.* 2020). Thus, the purpose of this experiment was to evaluate the photosynthetic light phase by JIP-test of chlorophyll *a* fluorescence in spiny cocklebur (*Xanthium spinosum* L.) plants as a noxious weed species present in corn (*Zea mays* L.), soybean (*Glycine max* L.), and some other summer crops after post-emergence application of nicosulfuron and bentazon herbicides.

Materials and Methods

Plant Materials and Growth Conditions

A pot experiment using a randomized complete block design with three replications was conducted in the glass greenhouse conditions under natural light (27-32 °C) in the University of Tabriz in 2017 to assess the effect of nicosulfuron and bentazon herbicides on spiny cocklebur (*X. spinosum* L.) chlorophyll *a* florescence (ChlF) parameters and PSII activity. Fifteen seeds of spiny cocklebur were sown in each plastic pot (20 × 20 cm) containing 1.0 kg of perlite at a depth of 1 cm and then tap water (0.6 dS m^{-1}) was added to achieve 100% field capacity. Plants were thinned to 5 plants per pot, after seedling establishment. The losses of pots were made up with Hoagland solution (Electrical conductivity =1.3 dS m^{-1} and pH= 6.5-7) and every 20 days, water was added to prevent further increase in the electrical conductivity. Herbicides were applied at the 4-5 leaf stage of spiny cocklebur at the recommended dose in the field as 2 $L.ha^{-1}$.

ChlF parameters from the upper surface of spiny cocklebur leaves were monitored with a handy-PEA portable fluorometer (Hansatech, UK) after 12, 36, 60 and 84 hours of herbicide application. This device has software for calculation, numerical presentation and memorization of chlorophyll *a* fluorescence parameters (Table 1).

All the data were analyzed based on the experimental design, using SAS 9.1 software. The means of each trait were compared using Duncan's multiple range test at $p \leq 0.05$.

Results

Application of nicosulfuron and bentazon increased initial fluorescence (F0) of plants. Bentazon had a higher effect on F0 at all times after application as compared with nicosulfuron. However, in most cases, especially at 12 and 84 hours after application of nicosulfuron, the effect of this herbicide on F0 was similar to the control treatment. Maximum F0 was observed at 36 hours after bentaznon application (Figure 1a). Maximum fluorescence (Fm) was only affected by bentazon and the effect of nicosulfuron on Fm was not significant. Fm significantly declined with increasing the time after bentazon application, so that the lowest amount of this variable was observed at 84 hours (Figure 1b).

Table 1. ChlF parameters measured on spiny cocklebur after herbicides application.

Abbreviations	Description
ChlF	Chlorophyll *a* fluorescence
PSI	Photosystem I
PSII	Photosystem II
PI	Performance index
ETC	Electron transfer chain
Q_A	Primary electron acceptor in PSII
F0	Initial fluorescence
Fm	Highest fluorescence
Fv	Variable fluorescence
Fv/Fm	The maximum quantum yield of PSII photochemistry
Fv/F_0	The activity of the water-splitting complex
Sm	The energy needed for the closure of reaction centers
Area	The area above the fluorescence induction curve between the minimum and maximum fluorescence for representing the size of the plastoquinone pool in photosystem II
Tfm	The time that takes to reach form F_0 to F_m, for representing the Quinone A reduction rate of the PSII acceptor
Sm/Tfm	The average redox state of Quinone A in the period from F_0 to F_m and concomitantly
VJ	*Fv* after 2 ms
VI	*Fv* after 30 ms
ABS/RC	Absorption energy flux in each reaction center
TR_0/RC	Trapped energy in each reaction center
ET_0/RC	Maximum electron transportation in each reaction center
DI_0/RC	Dissipation energy flux in each reaction center
φ_{E0}	Quantum yield for electron transportation from Quinone A^- to plastoquinone
ψ_{E0}	The probability that trapped excitation moves an electron into the electron transport chain beyond Quinone A^-
φ_{R0}	Quantum yield of reduction of end electron acceptors at the PSII acceptor side
K_N	The non-photochemical de-excitation rate constant in the excited antennae for non-photochemistry
K_P	The photochemical de-excitation rate constant in the excited antennae of energy fluxes for photochemistry

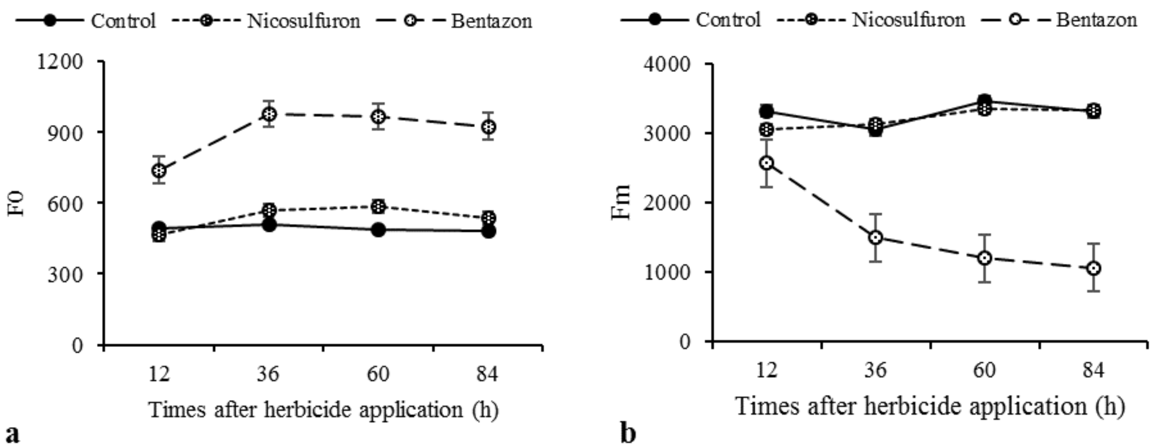

Figure 1. Changes in F0 (a) and F0 (b) of spiny cocklebur (*Xanthium spinosum* L.) after 12, 36, 60 and 84 hours of nicosulfuron and bentazon herbicides application.

Fv/Fm was not affected by nicosulfuron, while significantly decreased by bentazon. As the time after bentazon application increased, the amount of Fv/Fm declined, so that the lowest Fv/Fm was obtained at 84 hours after bentazon treatment (Figure 2a). Bentazon harmed Fv/F0, but nicosulfuron only slightly influenced this variable. Treated plants by nicosulfuron had lower Fv/F0 than that of control plants and this reduction started at 36 hours after the treatment. However, Fv/F0 decreased significantly with increasing the time after bentazon application. Thus, the minimum amount of Fv/F0 was observed at 84 h after bentazon application (Figure 2b).

Variable fluorescence (Fv) was reduced after

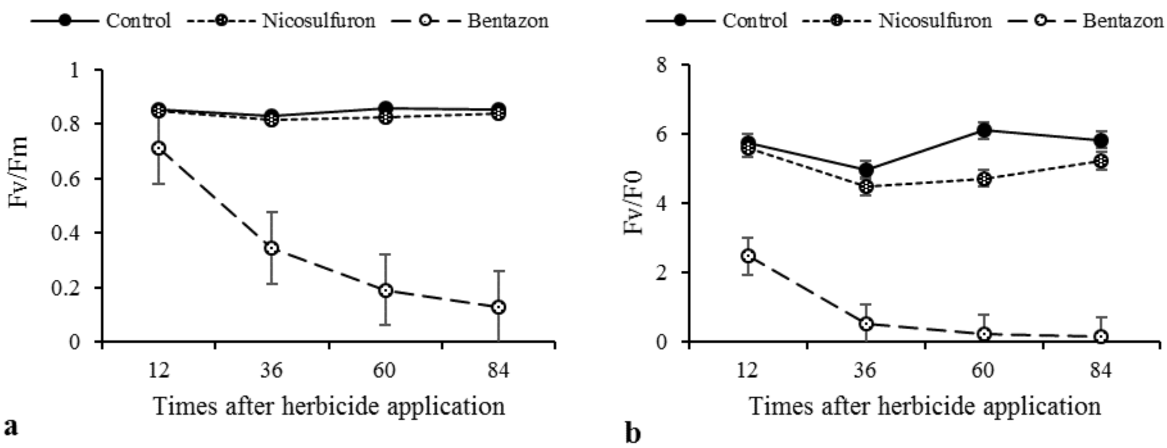

Figure 2. Changes in Fv/Fm (a) and Fv/F0 (b) of spiny cocklebur (*Xanthium spinosum* L.) after 12, 36, 60 and 84 hours of nicosulfuron and bentazon herbicides application.

bentazon application, and this reduction continued with increasing the time after the herbicide application (Figure 3a). *Area* slightly increased by the application of nicosulfuron, however, this increase was not significant at 12 and 60 hours after herbicide application. But, at 36 hours after bentazon application, *Area* decreased substantially (Figure 3b).

Sm and Sm/Tfm were significantly increased with the nicosufuron application. But, bentazon reduced Sm and it was not recovered after bentazon application (Figure 4a, b). Sm declined after 36 hours of the bentazon treatment and then remained unchanged (Figure 4a). However, the effect of bentazon on Sm/Tfm was not significant (Figure 4b).

The time taken to reach F_m (Tfm) was only affected by bentazon. Tfm in the bentazon treated plants at 12 hours after application was higher than that of other treatments; this variable was strongly reduced at 36 hours after herbicide application and then remained constant (Figure 5a). Photosynthesis relative vitality (PI) as an important chlorophyll *a* fluorescence parameter was strongly decreased by betnazon. PI in treated plants at 12 hours after bentazon application was close to zero and at 36 hours after this herbicide application had no activity. The negative effect of

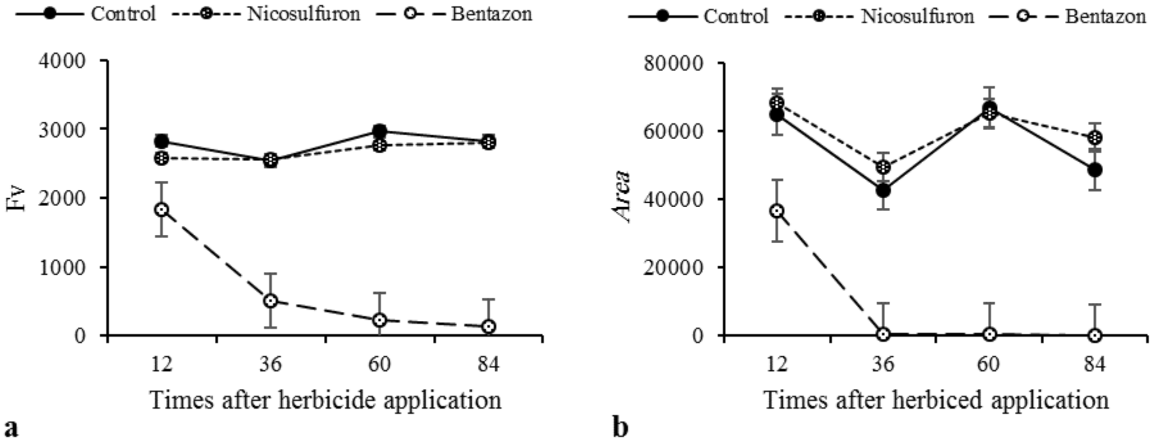

Figure 3. Changes in Fv (a) and *Area* (b) of spiny cocklebur (*Xanthium spinosum* L.) after 12, 36, 60 and 84 hours of nicosulfuron and bentazon herbicides application.

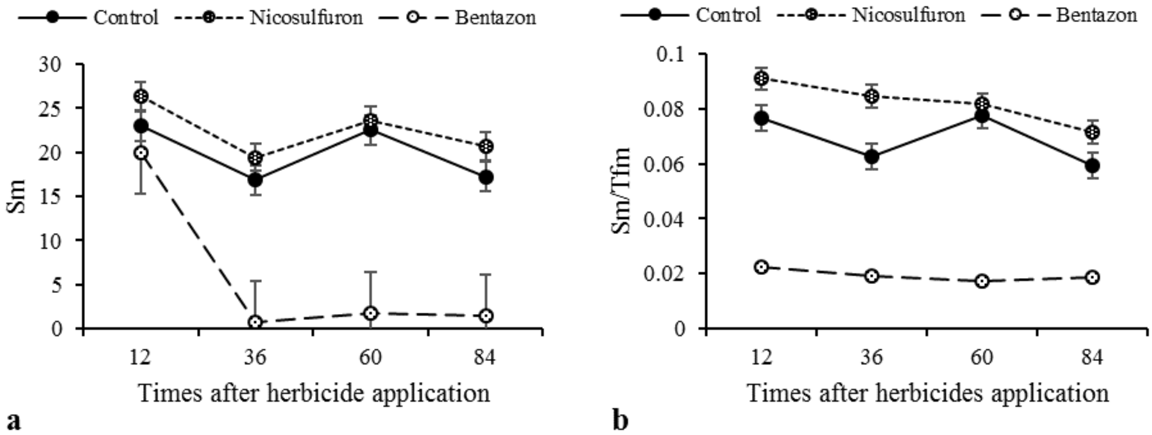

Figure 4. Changes in Sm (a) and Sm/Tfm (b) of spiny cocklebur (*Xanthium spinosum* L.) after 12, 36, 60 and 84 hours of nicosulfuron and bentazon herbicides application.

nicosulfuron on PI was observed at 36 hours after the herbicide application and after that time, this variable was not recovered significantly (Figure 5b).

Application of nicosulfuron after 12 h, caused a slight increase in K_P but, after 36 h, it decreased K_P in plants. However, application of bentazon after 12 h strongly decreased K_P, and this reduction was more evident with increasing the time after bentazon application (Figure 6a). Application of nicosulfuron did not affect K_N, however, bentazon strongly increased K_N after 12 h of application and this enhancement was greater at 84 h after the bentazon treatment (Figure 6b).

The effect of both nicosulfuron and bentazon herbicides on other ChlF parameters was presented as a spider plot graph in Figure 7. Bentazon increased Fv at 2 ms (Vj) and especially increased the absorption of ABS/RC, and with increasing the time after bentazon application, these effects were more pronounced. In contrast, this herbicide decreased ET_0/RC, ψ_{E0}, φ_{E0} and φ_{R0}.

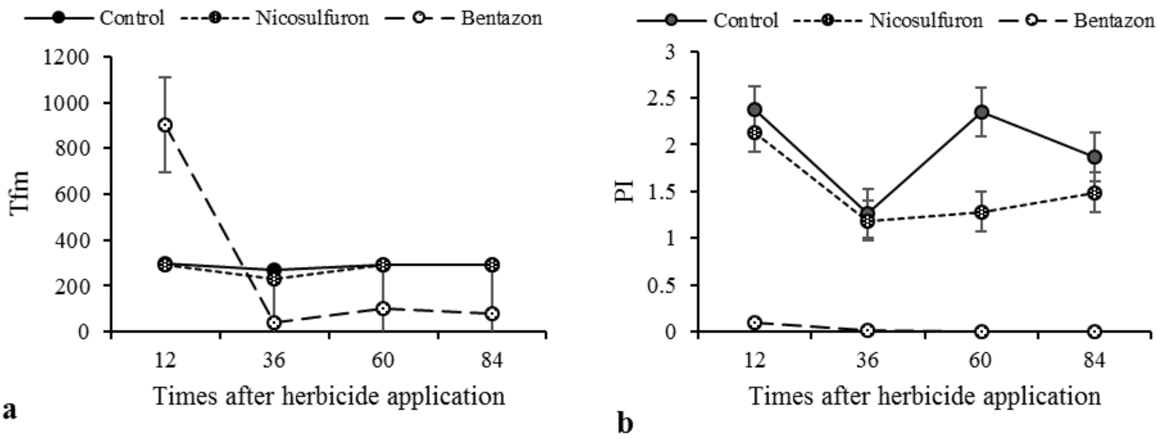

Figure 5. Changes in Tfm (a) and PI (b) of spiny cocklebur (*Xanthium spinosum* L.) after 12, 36, 60 and 84 hours of nicosulfuron and bentazon herbicides application.

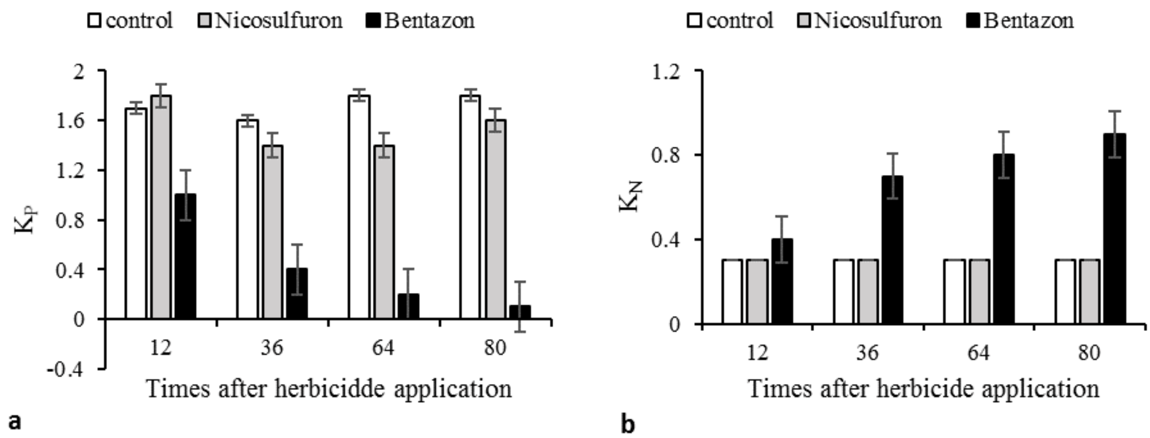

Figure 6. Changes in K_P (a) and K_N (b) of spiny cocklebur (*Xanthium spinosum* L.) after 12, 36, 60 and 84 hours of nicosulfuron and bentazon herbicides application.

However, the application of nicosulfuron did not affect the JIP parameters.

The lethal effects of an herbicide in higher plants depend on the particular side at which a physiological reaction is inhibited in a plant cell and its compartments. In the chloroplast, there are two main target sides of the herbicide action. One target is represented by the electron transport chain with its electron carriers and enzymes which are involved in phosphorylation and NADP photo-reduction. Another main target of the herbicide action is the biosynthesis of chlorophylls and carotenoids that are contained in the light-harvesting complex and the antennae of the photosynthetic reaction centers (Hassannejad *et al.* 2020; Baghbani *et al.* 2019). In our research application of bentazon in comparison with nicosulfuron strongly influenced PSII activity of spiny cocklebur plants. Bentazon enhanced F0 but decreased Fm of plants Figure 1). F0 is the

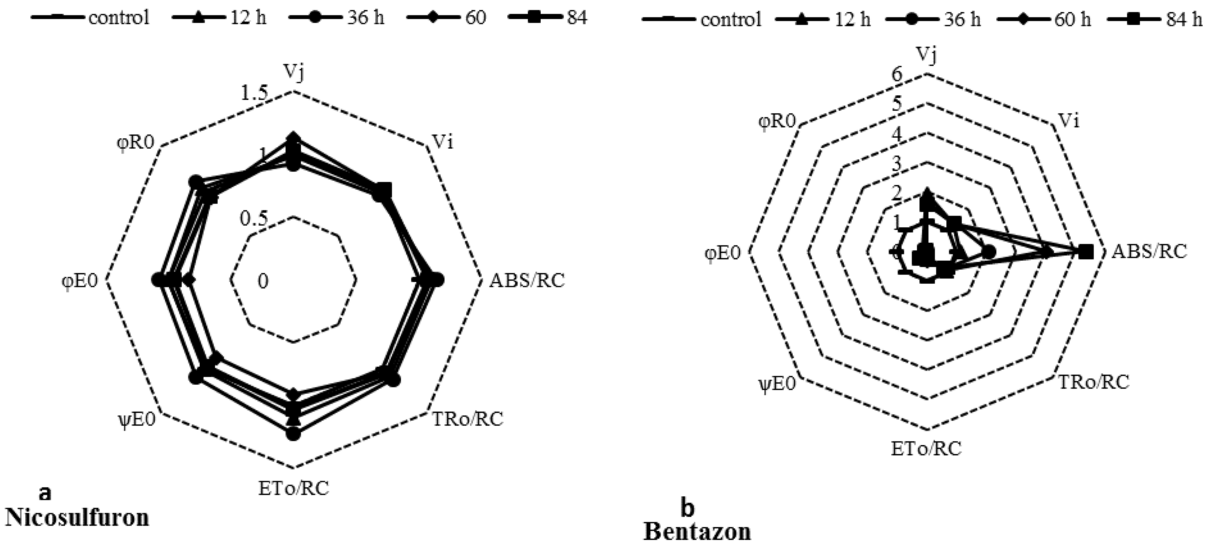

Figure 7. Spider plot presenting the JIP-test parameters calculated from nicosulfuron (a) and bentazon (b) treated spiny cocklebur (*Xanthium spinosum* L.) plants.

fluorescence level when plastoquinone (PQ) electron acceptor pool is fully oxidized and it may change upon exposure to stresses (Fracheboud *et al.* 2004). An increase in F0 can be interpreted as a reduction of the rate constant of energy trapping by PSII centers, which could be the result of a physical dissociation of light-harvesting complex from PSII core observed in several plant species under environmental stresses (Rong-hua *et al.* 2006). Reduction in Fm under bentazon application (Figure 1b) may have caused by the inhibition of electron transport at the donor side of the PSII (Hassannejad *et al.* 2020), which resulted in the Tfm decrease (Figure 5a).

Application of bentazon destructed the reaction centers of PSII in the treated plants (photo-chemically active), thus electron transport capacity in PSII and the number of quanta absorbed per unit time decreased. Bentazon treatment not only decreased protein kinase activity but also destructed D_1 protein level and correspondingly, Fv/Fm, electron transfer rate (ETR) and photosynthetic rate were reduced (Figure 2a). Fv/F0 is the most sensitive component in the photosynthetic electron transport chain (Hassannejad *et al.* 2020). The decrease in Fv/F0 (Figure 2b) as a consequence of bentazon application, results from photosynthetic electron transport destruction, which affects the Sm/Tfm parameter in plants (Figure 4b). Bentazon inhibits the electron transfer rate at the donor side of PSII and the electron transportation from reaction centers to the plastoquinone pool and decreased *Area* (Figure 3b). Decreasing in Sm (Figure 4a) was the consequence of a decrease in *Area* (Figure 3b) and Fv (Figure 3a) under application of bentazon. Application of bentazon impaired both light and dark reactions of photosynthesis as a result of the reduction in PI (Figure 5b). Decreasing in PI may be related to the effects of bentazon on the density of reaction centers per PSII antenna chlorophyll, maximum quantum yield for primary photochemistry and the quantum yield for electron transport (Hassannejad *et al.* 2020).

Bentazon strongly increased Vj and ABS/RC but decreased ET_0/RC, ψ_{E0}, φ_{E0} and φ_{R0} (Figure 7). As the ψ_{E0} values of bentazon samples were low, the electron carriers could not transfer electrons to the next step of the electron transport chain (Joshi *et al.* 1995; Toth *et al.* 2007; Lotfi *et al.* 2018; Hassannejad *et al.* 2020). After bentazon application, the PSII acceptor side was limited more than the PSII donor side as Lotfi *et al.* (2018) showed after humic acid application in rapeseed plants.

Conclusion

Bentazon belongs to the benzothiadiazinones and are known as PSII inhibitors and Nicosulfuron belong to the sulfonylurea a class of herbicides that by inhibiting the activity of acetohydroxyacid synthase/acetolactate synthase decreases the synthesis of branched-chain amino acids. According to the mode of action of the used herbicides, nicosulfuron did not affect the photosynthetic activity of the spiny cocklebur samples. However, bentazon strongly destroyed the photosystem II activity of spiny cocklebur. The donor side of PSII was more sensitive than its acceptor side to bentazon. This claim was supported by an increase in ABS/RC and a decrease in ψ_{E0} and φ_{E0}. After 12 h of bentazon application, the PI was close to zero and after 36 h the photosynthetic activity was completely destroyed.

Conflict of Interest

The authors declare that they have no conflict of interest with any organization in relation to the subject of the manuscript.

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چگونگی تغییرات فاز نوری فتوسنتز زردینه خاردار (*Xanthium spinosum* L.) در طی زمان بعد از کاربرد علفکش

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چکیده

فاز نوری گیاه زردینه خاردار (*Xanthium spinosum*) توسط آزمن JIP در زمان‌های مختلف (۱۲، ۳۶، ۶۰ و ۸۴ ساعت) بعد از کاربرد علف‌کش‌های نیکوسولفورون و بنتازون مورد مطالعه قرار گرفت. نتایج نشان داد که کاربرد نیکوسولفورون تاثیری روی بسیاری از پارامترهای فلورسانس کلروفیل a ندارد. اما، کاربرد بنتازون به طور معنی‌داری با افزایش فلورسانس حداقل و کاهش فلورسانس حداکثر، فلورسانس متغیر، فعالیت کمپلکس تجزیه کننده آب در بخش دهنده الکترون فتوسیستم II و به ویژه حداکثر عملکرد کوانتومی فتوشیمیایی فتوسیستم II، باعث کاهش فعالیت فتوسیستم II گردید. کاربرد بنتازون به دلیل افزایش جریان نوری جذب شده در هر مرکز واکنش و کاهش کارایی انتقال الکترون‌های به دام افتاده از مراکز واکنش به کوئینون A و نیز کاهش عملکرد کوانتومی انتقال الکترون از کوئینون A به پلاستوکوئینون، روی بخش دهنده الکترون فتوسیستم II به مراتب تاثیرگذارتر از بخش گیرنده الکترون آن بود. کاهش خاصیت حیاتی فتوسنتزی دستگاه فتوسنتزی توسط بنتازون، کلروفیل‌های مراکز واکنش فتوسیستم II، حداکثر عملکرد کوانتومی فتوشیمیایی اولیه و عملکرد کوانتومی برای انتقال الکترون را تحت تاثیر قرار داد. در بسیاری موارد، اثرات بازدارنده بنتازون روی فتوسیستم II، ۳۶ ساعت بعد از کاربرد علف‌کش گزارش شد. تیمار بنتازون سرعت زنجیره انتقال الکترون را کاهش و در پی آن سرعت بازانتشار فتوشیمیایی انرژی نورانی آنتن‌های برانگیخته را افزایش داد.

واژه‌های کلیدی: آزمون JIP؛ انتقال الکترون؛ بنتازون؛ زردینه خاردار؛ فلورسانس کلروفیل a؛ نیکوسولفورون.