

Research paper

Chlorophyll *a* fluorescence of common cocklebur (*Xanthium strumarium* L.) in response to Lumax herbicide with and without almond oil

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Abstract

Chlorophyll *a* fluorescence measurement is a useful tool for studying the herbicides' effects with different modes of action on the photosynthetic apparatus. In this research, the chlorophyll *a* fluorescence response of common cocklebur (*Xanthium strumarium* L.) plants was studied two days after the sole application of different doses of the Lumax herbicide (s-metolachlor + mesotrione + terbuthylazine), and combined with the almond oil. The quantum yield of energy dissipation (F₀/F_m) and minimum fluorescence (F₀) values of the plants treated with the 100% herbicide dose increased significantly from 0.16 to 0.25 and 296 to 391, respectively, two days after treatment. These changes in PSII resulted in the reduction of the maximum quantum yield of PSII (F_v/F_m) and the PSII function index (PI_{ABS}). Moreover, the application of high doses of the herbicide decreased the variable fluorescence (F_v), the maximum fluorescence (F_m), the size of the plastoquinone pool on the reducing side of photosystem II (Area), the maximum efficiency of water-splitting complex on the donor side of the photosystem II (F_v/F₀), the energy needed for the closure of reaction centers (S_m), and the intermediate redox state of quinone A in the period from F₀ to F_m. Our study revealed that the use of almond oil with the Lumax herbicide caused further reduction of the chlorophyll *a* fluorescence parameters in common cocklebur. The correlation between dry weight taken 28 days after the herbicide application and F_v/F_m measured two days after the herbicide application showed that assessing the chlorophyll *a* fluorescence parameters can speed up the rate of herbicide performance evaluation up to 12 times. The steeper slope of the regression line for herbicide with almond oil compared to the herbicide alone showed that almond oil could be a proper adjuvant to increase the herbicide efficiency.

Keywords: almond oil; common cocklebur; herbicide; photosynthetic apparatus; regression

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Introduction

The common cocklebur (*Xanthium strumarium* L.) is a nuisance weed in summer crops like cotton, corn, sunflowers, soybeans, and cotton (Hassannejad *et al.* 2020c). According to Hussain *et al.* (2014), corn yield reduced to 5-40% with as few as 10 common cocklebur plants per meter square. Because of its height and large leaves, this weed competes with the crop for light (Bosza and Oliver 1993).

Herbicides are among the easiest and the most

widespread agricultural tools in weed control due to their ease of use and high efficiency. Among all herbicides applied in crop protection, almost 50% impact the chloroplast (Cobb and Reade 2010). Lumax (mesotrione + s-metolachlor + terbuthylazine) is one of the most recently formulated pre-emergence herbicides known as the inhibitor of photosystem II (PSII) activity (Anorvey *et al.* 2018). Lumax (537.5 SE) is used to control annual grasses and broadleaf weeds by interfering with normal germination and seedling

development. This herbicide is a combination of three s-metolachlor (0.375 kg a.i./L), mesotrione (0.0375 kg a.i./L), and terbuthylazine (0.125 kg a.i./L) herbicides with different action sites that prevent pigment synthesis and photosynthesis in photosystem II (Hassannejad *et al.* 2020a).

Chlorophyll fluorescence examination is one of the most effective and widely used techniques to study the effects of different stresses, including the herbicide stress in plants (Guidi *et al.* 2019). Using some herbicides in plants stops the electron transfer chain due to damage to the photosynthetic apparatus, in which chlorophyll performs fluorescence to return to a steady-state (Kalaji *et al.* 2017). Chlorophyll fluorescence has been routinely used to survey the photosynthetic apparatus of plants treated by herbicides with a different mode of action, such as glyphosate (Zanata *et al.* 2020), graminicides (Matzrafi *et al.* 2017), and phenoxy herbicides (Gutierrez *et al.* 2020). It also provides an insight into the potential impacts of different herbicides on metabolism, translocation, detoxification, and light-dependent reactions of photosynthesis (Kumar *et al.* 2014), which confer resistance to the weeds (Juneau *et al.* 2001).

Plant oils are environmental friendly, biodegradable by soil microorganisms, and more valuable than paraffinic petroleum oils (Cornish *et al.* 1993; Cabanne, 2000). Investigations show that some vegetable oils can enhance the efficacy of several herbicides on some weeds (Izadi Darbandi *et al.* 2013; Izadi Darbandi and Aliverdi 2015). It is possible to increase the effectiveness of herbicides by adding plant oils to the spray tank (Rastgoo *et al.* 2020). Almond oil has been used to enhance the phytotoxicity of pinoxaden and

haloxyfop-R-methyl on *Phalaris minor* Retz. (Rastgoo *et al.* 2020).

The purpose of this study was to assess the efficacy of the Lumax herbicide with and without almond oil on photosynthetic parameters measured via chlorophyll fluorescence in common cocklebur as one of the main weeds in summer crops.

Materials and Methods

This study was conducted to evaluate the chlorophyll fluorescence of the common cocklebur plants in response to 25, 50, 75, and 100% of the recommended dose (4.5 liters per hectare) of Lumax [s-metolachlor (0.375 kg a.i./L), mesotrione (0.0375 kg a.i./L), terbuthylazine (0.125 kg a.i./L)] with and without almond oil at 0.3 (v/v). A pot experiment using a randomized complete block design with three replications was conducted in the glasshouse conditions of the University of Tabriz in 2017, under 14 hours of natural light (27-32 °C). Germination of common cocklebur achene requires daily exposure to natural light at 24 to 30 °C (Norsworthy and Oliveira 2007). Seeds (achenes) of the common cocklebur plants were collected from the mature plants in the research field of the University of Tabriz and were placed in the open air for three months during the winter to break the seed dormancy. Fifteen seeds of common cocklebur were grown in in the depth of 3 cm in plastic pots (20 × 20 cm × 25 cm) filled with 1 kg of perlite and then tap water (0.6 dS m⁻¹) was added to achieve 100% field capacity. At the three-leaf stage, plants were thinned to 5 plants per pot. We used the Hoagland solution (electrical conductivity= 1.3 ds m⁻¹ and pH= 6.5-7) to make up the nutrients' loss in pots (Hassannejad *et al.*

2020b). Every 20 days, water was added to prevent the further increase in electrical conductivity (Hassannejad *et al.* 2020b). Herbicide was applied once using a laboratory pot sprayer equipped with a flat-fan nozzle operating at a pressure of 3 bars and a velocity of 4 km h⁻¹, delivering a spray volume of 200 L ha⁻¹ on the upper side of the common cocklebur leaves after the appearance of 4 to leaves (Hassannejad *et al.* 2020b).

Chlorophyll fluorescence was monitored from the upper surface of the fully expanded common cocklebur leaves using a plant efficiency analyzer (Handy PEA fluorimeter, Hansatech instruments Ltd. England) 1, 2, 3, and 4 days after the herbicide treatment. However, the changes occurred two days after the herbicide application and no significant changes were observed after the third and fourth days. Therefore, the second-day data were used for the data analysis. Before measuring the chlorophyll fluorescence signals, the dark-

adapted leaves (30-40 min) were exposed to solid actinic light (3000 μmol photons/m²s) and fluorescence was recorded from 20 μs to 2 s (Hassannejad and Porheidar Ghafarbi 2018; Abbasvand *et al.* 2019). After that, the chlorophyll fluorescence signals were exported with the PEA plus (1.10) software (Hassannejad *et al.* 2020c).

The recorded data were analyzed using SAS 9.1 and Microsoft Office Excel 2020 software based on the experimental design. The means for each trait were compared according to Duncan's multiple range test at $p \leq 0.05$.

Results

Area

The use of different doses of the Lumax herbicide did not change the area (the size of the PQ pool on the reducing side of PSII) (Figure 1). In contrast, the addition of almond oil to herbicide significantly decreased this parameter (Figure 1).

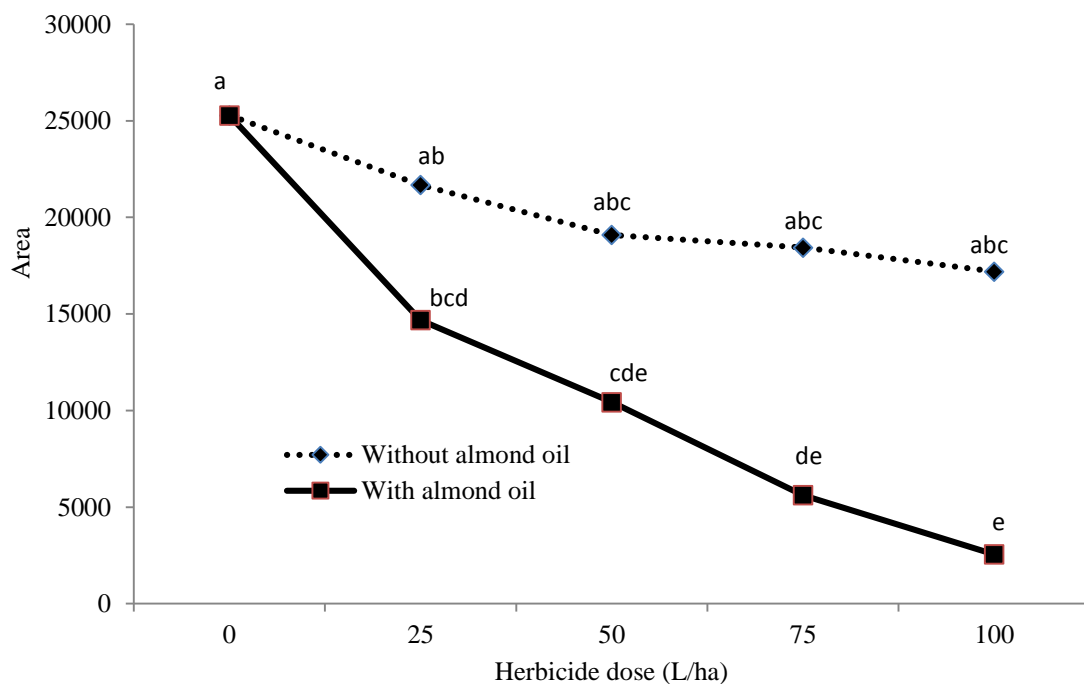


Figure 1. Changes in the area (the size of the PQ pool on the reducing side of PSII) of common cocklebur in response to 0, 25, 50, 75, and 100% of the Lumax (mesotrione + s-metolachlor + terbuthylazine) recommended dose and almond oil application. Each value is the mean of three replicates (Duncan's multiple range test; $p \leq 0.05$).

F0 and Fm

In this experiment, the minimum fluorescence (F0) increased significantly by using a 100% dose of the Lumax herbicide, which could be represented as a changeover part of the active PSII reaction centers (RC) into a form of silent RC (Figure 2a). The use of almond oil had no significant effect on reducing

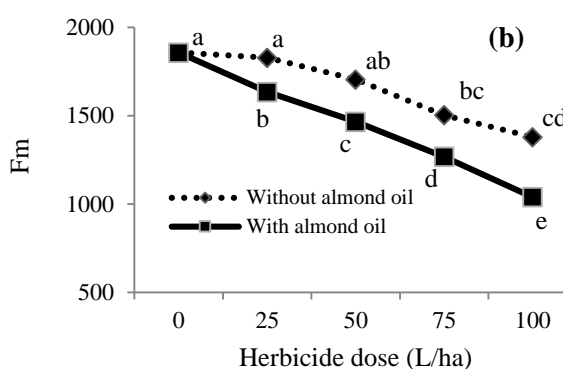
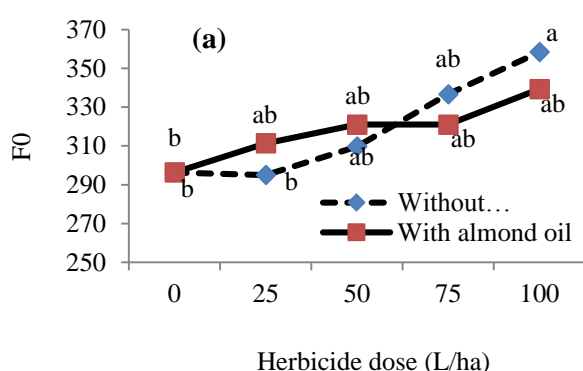


Figure 2. Changes in F0 (a) and Fm (b) of common cocklebur in response to the Lumax (mesotrione + s-metolachlor + terbuthylazine) recommended dose and almond oil application. Each value is the mean of three replicates (Duncan's multiple range test; $p \leq 0.05$).

Fv and Fv/Fm

The variable fluorescence (Fv) in plants treated with Lumax was slightly reduced by increasing the herbicide dose. This reduction was increased by adding the almond oil to the Lumax herbicide and reached about 34.18% reduction at 100% dose of the herbicide as compared to the control (Figure 3a). Two days after the herbicide treatment, the maximum quantum efficiency of PSII (Fv/Fm) of plants treated with 25% and 50% dose of Lumax was the same as that for the control but decreased in 75% and 100% dose of this herbicide (Figure 3b). The addition of almond oil to Lumax significantly reduced the efficiency of the photosystem when a high dose of herbicide (100%) was applied (Figure 3b).

or increasing the effectiveness of the herbicide in terms of the F0 parameter (Figure 2a). With increasing the dose of herbicide, the maximum fluorescence (Fm) had a downward trend. The addition of almond oil to the herbicide composition increased the rate of this decrease (Figure 2b).

Fv/F0 and F0/Fm

The maximum efficiency of the water-splitting complex on the donor site of the PSII (Fv/F0) declined under all doses of the herbicide. However, this reduction was more severe at higher doses of the herbicide application. This reduction increased by adding the almond oil to the herbicide composition (Figure 4a). As shown in Figure 4b, F0/Fm increased with increasing the dose of Lumax as compared with the control, but this increase was not significant at the 25% dose of herbicide application (Figure 4b). Adding almond oil to the herbicide made this increment more pronounced (Figure 4b).

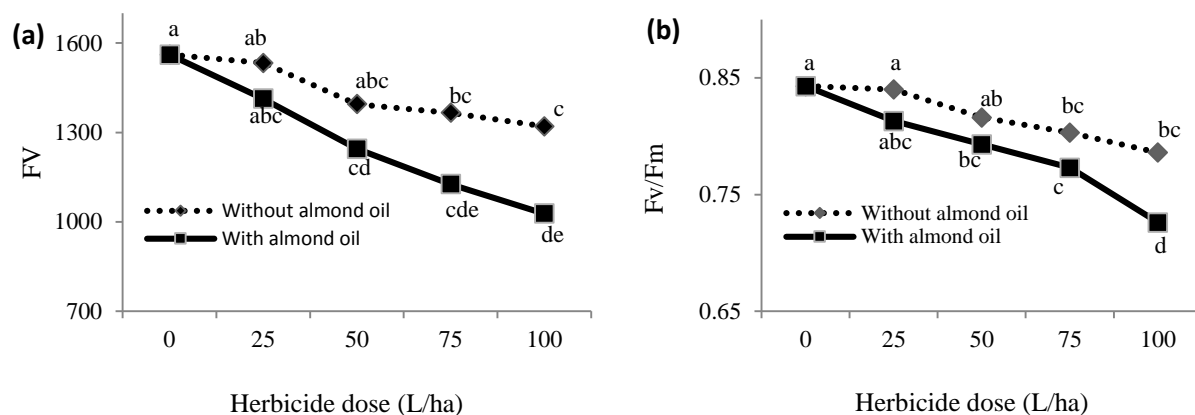


Figure 3. Changes in Fv (a) and Fv/Fm (b) of common cocklebur in response to the Lumax (mesotrione + s-metolachlor + terbuthylazine) recommended doses and almond oil application. Each value is the mean of three replicates (Duncan's multiple range test; $p \leq 0.05$).

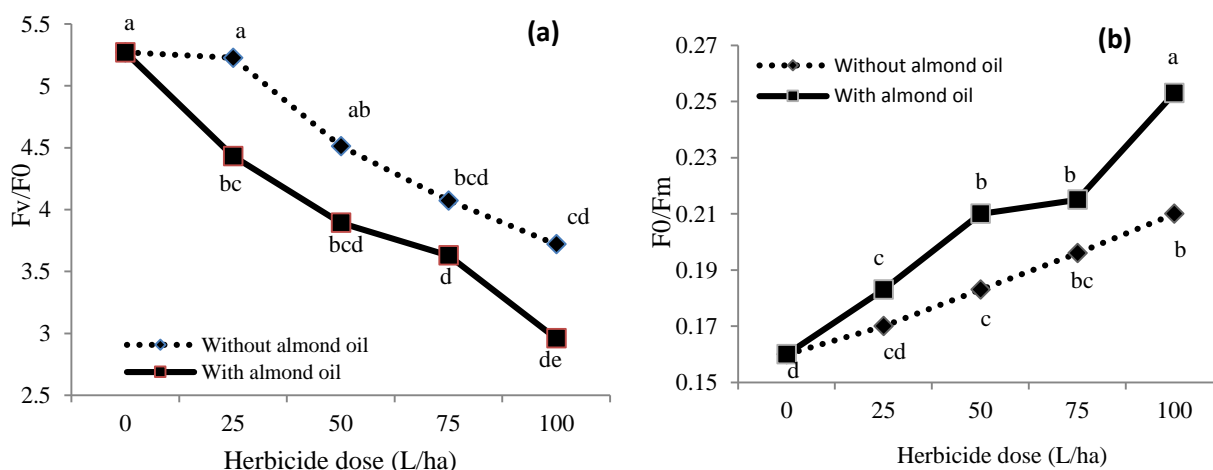


Figure 4. Changes in Fv/F0 (a) and F0/Fm (b) of common cocklebur in response to the Lumax (mesotrione + s-metolachlor + terbuthylazine) recommended dose and almond oil application. Each value is the mean of three replicates (Duncan's multiple range test; $p \leq 0.05$).

PI_{ABS} and PI_{total}

Performance index (PI_{ABS}) is calculated on an energy absorption basis. The value of PI_{ABS} was lower in the plants treated with high doses of herbicide (Figure 5a). However, this reduction did not change significantly with the addition of almond oil to the herbicide composition, except at

the 25% dose (Figure 5a). The use of 75% and 100% doses of Lumax reduced the PI_{total} , which measures the performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors, and the addition of almond oil to the herbicide had a synergistic effect on this reduction trend (Figure 5b).

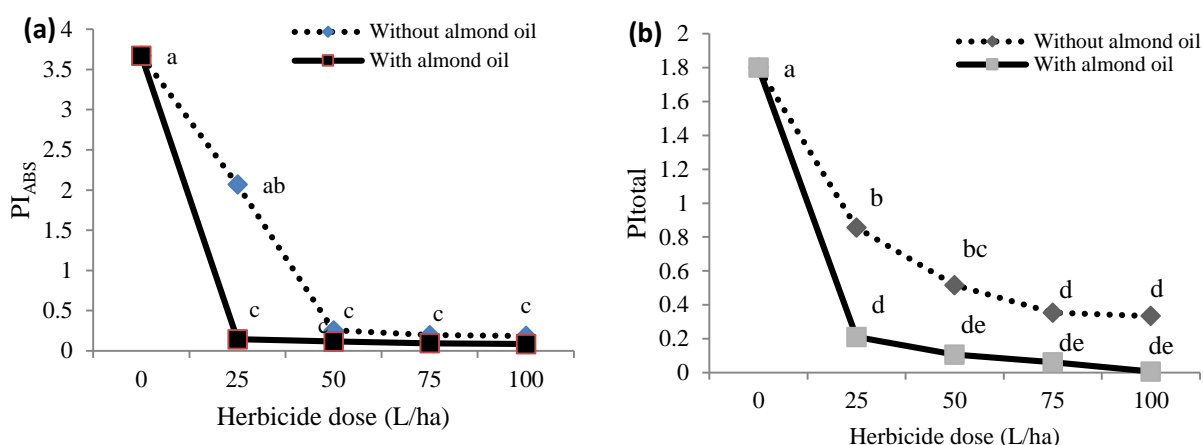


Figure 5. Changes in PI_{ABS} (a) and PI_{total} (b) of common cocklebur in response to the Lumax (mesotrione + s-metolachlor + terbuthylazine) recommended dose and almond oil application. Each value is the mean of three replicates (Duncan's multiple range test; $p \leq 0.05$).

Sm and Sm/Tfm

Complementary area (Sm) decreased about 21.24% by using a 100% dose of the herbicide; however, this decrease was much higher in the almond oil-treated plants than the sole herbicide-treated plants (Figure 6a). The middle fraction of QA in the period from 0 to Tfm (Sm/T_{fm}) was

similar in the control and 25% dose of Lumax. However, it was dramatically decreased by increasing the herbicide dose (Figure 6b). The use of almond oil with herbicides had a synergistic effect and increased the rate of this decrease (Figure 6b).

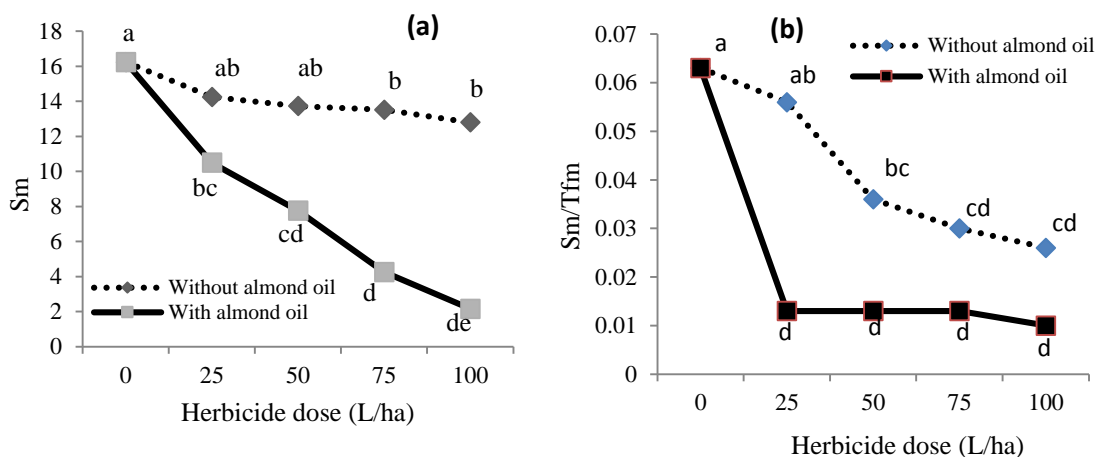


Figure 6. Changes in Sm (a) and Sm/T_{fm} (b) of common cocklebur in response to the Lumax (mesotrione + s-metolachlor + terbuthylazine) recommended dose with and without almond oil application. Each value is the mean of three replicates (Duncan's multiple range test; $p \leq 0.05$).

The relationship between Fv/Fm and dry weight

Figure 7 illustrates the relationship between Fv/Fm, taken two days after the Lumax application, and dry weight at 28 days after the

Lumax application with and without almond oil. A linear regression of Fv/Fm with dry weight was evident. The slope of the regression line was steeper for Lumax with almond oil as compared to

Lumax alone. Other researchers also reported a relationship between Fv/Fm and dry weight for bentazone (Christensen *et al.*, 2003), metamiltron and terbuthylazine (Abbaspoor *et al.* 2006),

clodinafop (Abbaspoor and Streibig, 2005), desmedipham and phenmedipham (Abbaspoor and Streibig, 2007), and sethoxydim with or without turnip oil (Hammami *et al.* 2014).

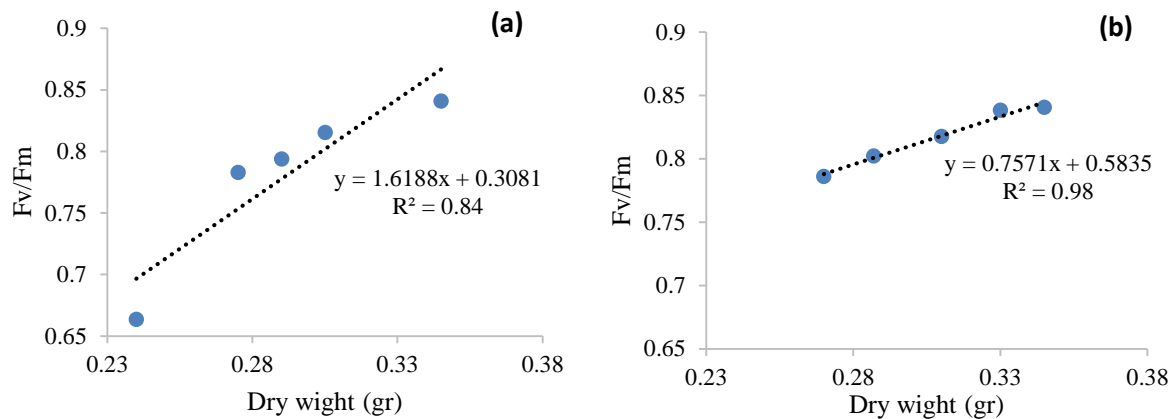


Figure 7. The relationship between Fv/Fm (the maximum quantum yield of photochemistry), taken two days after the Lumax application with dry weight, taken at 28 days after the Lumax application with (a) and without (b) almond oil in common cocklebur

Discussion

The application of high doses of Lumax (75% and 100% of the recommended dose) destroyed the PSII reaction centers of common cocklebur. It reduced the electron transport capability in PSII by disturbing the number of quanta absorption per unit time. F0 is the fluorescence level when all primary quinone acceptors (QA) are in the oxidized state (open), and it increases when the plant is exposed to stress (Kumar *et al.* 2020). Plants exposed to 25% and 50% of the recommended herbicide dose without almond oil showed no considerable effect on Fm. At the same time, a severe reduction in this parameter was observed after adding almond oil to the herbicide spraying tank (Figure 2b). The decrease in Fm when almond oil was added to the herbicide (Figure 2b) may be caused by the reduction of photosystem II activity, which results from the inhibition of electron transport at the donor side of PSII (Hassannejad *et al.* 2020c). Decreasing area (Figure 1) and Sm (Figure 6a) by adding almond oil to the Lumax herbicide showed that the PQ pool of reduced electron acceptors in PSII (mainly QA) is small, and electron flow at the donor site of the PSII was inhibited.

Fv/F0 ratio reduction as a result of Lumax and almond oil application (Figure 4a) showed a lower efficiency of the water-splitting complex on the donor side of PSII (Kalaji *et al.* 2018). This represents a donor side photoinhibition mechanism by malfunction of the water-splitting complex (Dayan *et al.* 2012) that may result in harmful oxidation in PSII (Zhang *et al.* 2016). This reduction in Fv/F0 might be due to the decrease in the PSII performance index (PI_{ABS}), which describes the energy absorption basis and disrupts water photolysis in PSII (Figure 5a). In most plants, the optimal values of maximum quantum yield of PSII (Fv/Fm) in normal conditions are about 0.83 (Kalaji *et al.* 2011). So, the values lower than that by applying high doses of Lumax may even be attributed to the reduced capability of PSII to transport electrons under herbicide stress. These results were similar to the earlier study by Hassannejad *et al.* (2020a). This research demonstrates that Lumax causes excess excitation energy accumulation in the PSII reaction center and significantly reduces the Fv/Fm in common cocklebur (Figure 3b).

The photosynthetic performance index (PI_{ABS}) that is used to evaluate the effects of environmental constraints on plants and correlates well with the plant vitality, decreased significantly in the plants treated with high doses of the Lumax herbicide (50, 75, and 100%) (Figure 5a). The decline of PSII activity and the loss of PSI structure and function causes a decrease in PI_{ABS} . PI_{total} , that was used to measure the performance of the reduction of PSI ends electron acceptors, declined in the herbicide and almond oil-treated plants (Figure 5b). The values of PI_{total} describe the energy flow efficiency of the photosynthetic transport chain beyond PSII (Strasser *et al.* 2010). This parameter is the outcome of the performance index on absorption basis for PI_{ABS} , and is a valuable parameter for monitoring herbicide treatments.

Conclusion

The impact of Lumax alone and combined with almond oil on the chlorophyll fluorescence of common cocklebur was presented in this research paper. Our findings confirmed that using high doses of Lumax intensively destroyed the photosystem II activity of common cocklebur plants by disrupting the water photolysis site and reducing the size of the PQ pool. It was found that combining almond oil with the Lumax herbicide has a synergistic effect and causes severe

damage to the plant's photosynthetic apparatus and chlorophyll parameters. Traditional screening methods can be replaced by the chlorophyll fluorescence studies based on the existing data and experimental results. Because, when compared to other screening procedures, the chlorophyll fluorescence approach is non-destructive, highly sensitive, and quick. Fv/Fm was shown to be a valuable metric for measuring the effect of herbicides quickly after spraying in this study. Furthermore, this study found that traditional screening procedures take 12 times longer than the chlorophyll fluorescence method. As a result, the chlorophyll fluorescence approach in the herbicide bioassay research may be expanded in the future. Finally, the relationship between dry weight and Fv/Fm could be exploited to increase the effectiveness of the herbicide screening trials.

Acknowledgment

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Conflict of interest

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

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فلورسانس کلروفیل *a* توق (*Xanthium strumarium* L.) در پاسخ به اختلاط علف کش ترکیبی لوماکس با و بدون روغن بادام

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چکیده

اندازه‌گیری فلورسانس کلروفیل *a* ابزاری مناسب برای مطالعه اثر علف‌کش‌ها با نحوه عمل متفاوت روی دستگاه فتوسنتزی است. در این آزمایش، پاسخ‌های فلورسانس کلروفیل گیاهان توق (*Xanthium strumarium* L.) دو روز بعد از کاربرد دزهای مختلف علف‌کش لوماکس (اس-متالاکلر + مزوتریون + تربوتیلازین) به تنهایی و در ترکیب با روغن بادام مورد مطالعه قرار گرفت. مقادیر عملکرد کوانتومی هدررفت انرژی (F0/Fm) و حداقل فلورسانس (F0) گیاهان تیمار شده با دز ۱۰۰ درصدی علف‌کش در دو روز بعد از اعمال تیمار به شدت افزایش یافته و به ترتیب از ۰٫۱۶ به ۰٫۲۵ و از ۲۹۶ به ۳۹۱ رسید. این تغییرات در فتوسیستم دو در نتیجه کاهش حداکثر عملکرد کوانتومی فتوسیستم دو (Fv/Fm) و شاخص کارایی فتوسیستم دو (PIABS) است. علاوه بر این، کاربرد دزهای بالای علف‌کش باعث کاهش فلورسانس متغیر (Fv)، حداکثر فلورسانس (Fm)، حجم کوئینون‌ها در بخش احیای فتوسیستم دو (Area)، حداکثر کارایی کمپلکس تجزیه‌کننده در سمت دهنده الکترون فتوسیستم دو (Fv/F0)، انرژی موردنیاز برای بستن مراکز واکنش (Sm) و متوسط اکسیداسیون و احیای کوئینون A در بازه زمانی از F0 تا Fm شد. در نهایت، این مطالعه نشان داد که استفاده از روغن بادام با علف‌کش لوماکس باعث کاهش عمده پارامترهای فلورسانس کلروفیل *a* در توق شد. همبستگی بین وزن خشک اندازه‌گیری شده ۲۸ روز بعد از کاربرد علف‌کش و Fv/Fm ارزیابی شده ۲ روز بعد از کاربرد علف‌کش نشان داد که با ارزیابی سریع پارامترهای فلورسانس می‌توان سرعت بررسی‌های مربوط به اثربخشی علف‌کش را تا ۱۲ مرتبه افزایش داد. شیب تند خط رگرسیون علف‌کش با روغن بادام در مقایسه با علف‌کش تنها نشان داد که روغن بادام می‌تواند به عنوان یک ماده افزودنی کارایی علف‌کش را افزایش دهد.

واژه‌های کلیدی: توق؛ دستگاه فتوسنتزی؛ رگرسیون؛ روغن بادام؛ علف‌کش