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Can Herbicide Stress in Artichoke (*Cynara cardunculus*) be Detected by Chlorophyll Fluorescence?

Sajid Ali[1,2*] and Bernd Honermeier[2]
[1]*Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan*
[2]*Institute of Agronomy and Plant Breeding I, Justus-Liebig University, Giessen, Germany*
*For correspondence: sajid.iags@pu.edu.pk

Abstract

Artichoke leaves contain a high percentage of polyphenols that are used for certain medicinal purposes. Due to wide row spaces and slow plant development during early growth stages, there is a high risk of weed infestation in field cultivation of artichoke. Post emergence herbicides used for weed control may also affect the non target crop by a temporary or permanent stress. Abiotic stress in crops may be detected by certain parameters like measurement of chlorophyll fluorescence after the application of herbicides. A field experiment was carried out at the research station Giessen, Germany during 2008 to study the possibility of herbicide stress detection in artichoke by measuring chlorophyll fluorescence. Eight post-emergence herbicides i.e., Phenmedipham, Pyridate, Quizalofop-P, Prosulfocarb, Carfentrazone, Rimsulfuron, Aclonifen and Clomazone were used in comparison with a mechanically weeded control. Fluorescence yield of light and dark adapted leaves showed that Clomazone affected chlorophyll fluorescence at one day after herbicide application and the effect went on increasing followed by that of Aclonifen and Rimsulfuron (appeared one week after herbicide application). These adverse effects started decreasing from three weeks after application of herbicides and were almost normal (compared with that of control) at four weeks after herbicide application during first growth phase, whereas these showed no recovery during second growth phase. Experimental study led to the conclusion that different post-emergence herbicides, depending on their mode of action, affect leaf yield of artichoke and chlorophyll fluorescence measurement can be used to detect the herbicide stress in artichoke leaves under field conditions. It can also be concluded that artichoke can recover this stress along with the development stages depending on the severity of herbicide stress and prevailing environmental conditions.

Keywords: Herbicide stress, *Cynara cardunculus* L., Chlorophyll fluorescence, Growth, Leaf yield

Introduction

Artichoke (*Cynara cardunculus* L.) is a cross-pollinated and highly heterozygous plant of family Asteraceae. It is an herbaceous perennial plant well adapted to xerothermic conditions of Southern Europe (Moglia *et al.*, 2008; Raccuia *et al.*, 2004) and typical conditions of arid and semi-arid areas of the Mediterranean environment (Raccuia *et al.*, 2004; Gominho *et al.*, 2001). Italy is the world's leading artichoke producer with a production of about 480112 metric tons artichoke per year (FAO, 2013). In Germany, artichoke is used for medicine production and dietary supplements since the 70's. Leaf extract of artichoke has been used for a variety of purposes, including chronic albuminuria, hyperlipidimia, irritable bowl syndrome (IBS), jaundice and liver dysfunction. Artichoke leaves have been used for recovery against abdominal pain, bloating, flatulence and nausea in 4-6 weeks with a low rate of side effects. It has also been used as diuretic adjuvant and to manage postoperative anemia. Artichoke leaves are known for their therapeutic potential as diuretic, choleretic, anti-diabetic and anti-microbial agent particularly in folklore (Krizkova *et al.*, 2004).

Due to its rosette growth nature, artichoke needs wide inter and intra row spaces. Rosette growth nature and slow development of juvenile plants may stimulate emergence and growth of weeds, which must be controlled by mechanical, chemical or biological methods. Application of selective (non-toxic) herbicides is the most effective method of weed management in artichoke. Different pre- and post-emergence herbicides are recommended and used for the control of weeds in artichoke. Post emergence herbicides may impose a stress to non-target crop (artichoke) either by making a layer on the leaves that may inhibit photosynthesis, affect the activity of stomata, positioning of leaves and fungal attack or may affect one of the metabolic or physiological functions (Varshney *et al.*, 2012; XiaoWen *et al.*, 2010). This stress may be temporary or permanent as the artichoke can cover it in some time.

Herbicides possibly can have adverse effect on fluorescence ability of leaves as these may alter the chlorophyll content of the leaves, or may produce a layer on the leaves, which may lead to low photosynthetic yield. It is an established fact that herbicides may affect plant's

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physiological state by inhibiting photosynthesis or associated biochemical processes (Krause and Weis, 1984). Derived from the use of photosynthetic parameters of chlorophyll fluorescence measurements derived from the use of photosynthetic parameters have been widely used in in photosynthetic physiology, eco-physiology, stress physiology, and agriculture. While in many cases the focus is primarily photosynthesis in other chlorophyll fluorescence is used as a general non-destructive, non-contact and convenient probe of a biotic stress (Baker, 2008). Post-emergence herbicides can affect the chlorophyll content of the leaves as these can cause chlorosis, necrosis on the leaf area and hence decrease the green color of the leaves. That is why plant biochemical parameters linked to photosynthesis such as ATP-formation, CO_2 fixation and O_2-evolution have been used as reliable indicators for herbicide and other pollutant effects (Wong and Couture, 1986). Herbicides may enter plants through roots, leaves or both, but in each case they are designed to control weeds (non-desirable plants) by inhibiting photosynthesis or by altering other metabolic processes (Tomlin, 2000). Herbicides, depending on their effects on the photosynthetic processes, are divided into two groups i.e., herbicides affecting photosynthetic electron transport and the herbicides affecting cellular metabolic processes not directly linked to photosynthetic electron transport. Chlorophyll fluorescence analysis is a sensitive and early indicator of damage to photosynthetic apparatus (Krause and Weis, 1991; Schreiber *et al.*, 1994).

Previous studies have shown that measurement of chlorophyll *a* (Chl *a*) fluorescence in plants is an efficient tool for studying photosynthesis. It has been established, after thorough studies, that dissipated chlorophyll fluorescence associated to photosynthesis can be used as a simple and rapid method to investigate the plant physiological state (Krause and Weis, 1984; Lichtenthaler and Rinderle, 1988). As many herbicides affect photosynthetic processes, chlorophyll fluorescence offers provides higher sensitivity compared to that of, for instance, bio-tests based on inhibition of growth (El Jay *et al.*, 1997). Some previous studies have reported the advantages of using chlorophyll fluorescence as a bio-test (Baker, 2008).

For the last two decades PAM-fluorometry has been used as a diagnostic tool to study the herbicide effects on photosynthesis. This method has an important advantage, as it is possible to assess the change of energy dissipation pathways by the exposure of the plants to the herbicide toxic effects. Different fluorescence parameters can be obtained by using PAM-fluorometry and can be used as indicators of toxicity.

Keeping the importance of artichoke and herbicides in view, present research project was carried out to study that, Is there any adverse effect of post emergence herbicides on artichoke (non-target crop)?, what is the duration of this stress and Can the stress imposed by post emergence herbicides be detected by non-destructive chlorophyll fluorescence measurements?

Materials and Methods

A field experiment was carried out at experimental research station of the Institute of Agronomy and Plant Breeding I in Giessen (Germany). The experimental site is situated at latitude of 50° 36´ North and a longitude of 8° 39´ East and an altitude of 158 meter above sea level. Geologically the soil belongs to alluvial origin. Top 30 cm layer of the soil is crumb, which is silt loam in nature. Soil nutrient status consisted of 34.0 kg ha^{-1} mineral N (NO_3^-- N + NH_4^+-N in 0 – 90 cm soil depth), 14.9 mg 100 g^{-1} P, 6.41 mg 100g^{-1} K, 27.4 mg 100 g^{-1} Mg with a pH value of 6.5. 80 kg N ha^{-1} was applied as basal dose at the time of seedbed preparation. Weather data at the experimental station Giessen, Germany for the growth period of artichoke (April to October) is presented in Fig. 1.

The herbicide experiment was carried out in randomized complete block design (RCBD) with four replications. Gross plot size was maintained as 3.0 m × 7.0 m and net plot measured to be 1.5 x 7.0 m^2, where 25 cm plant-to-plant distance was maintained in 75 cm spaced rows. Gobbo di Nizza cultivar of artichoke (cardoon) was sown manually at approximately 2 cm soil depth in April, 2008. Eight post emergence herbicides with different mode of actions (Table 1) were used against a manually weeded control (Table 2).

Chlorophyll fluorescence data were recorded by pulse amplitude modulation (PAM) technique by the use of a portable chlorophyll fluorometer Mini PAM (H. WALZ, Germany). Four plants were randomly selected from the middle rows of each plot and the tip area (4–5 cm) of any of two youngest leaves was used for chlorophyll fluorescence measurement. Leaf clip was used to hold the leaf and an impulse of light was passed from the instrument through the leaf. The effective quantum yield of photosynthese II (ΔF/Fm) was calculated as (Fm-Ft)/Fm, where Fm is maximum fluorescence and Ft is ground/Zero fluorescence.

Chlorophyll fluorescence (µmol CO_2 m^{-2} s^{-1}) was recorded automatically in the Mini-PAM, and transferred to computer by means of 'Pamtrans' software. The average of the four measurements was worked out for further manipulation. Chlorophyll fluorescence data were recorded for both light and dark-adapted plants at one day, one week, two weeks, three weeks and four weeks after herbicide application. Light adapted plants measurements were made under direct sunlight whereas for dark-adapted measurements the plants were covered with dark sheets for four minutes and then measurements were made under this sheet.

Statistical package SAS was used for checking the significance of the different treatments. 5% probability level was used for studying the difference between experimental treatments. Least significance difference (LSD) test was used to compare different treatment means. The standard deviations (SD) in figures were calculated by using Microsoft Excel.

Table 1: Classification of the herbicides used

Trade Name	Active Ingredient	Chemical Family	Mode of Action	HRAC/WSSA Group
Kontakt 320 SC	Phenmedipham	Phenyl-carbamate	Inhibition of photosynthesis at Photosystem II	C1/5
Lentagran WP	Pyridate	Phenyl-pyridazine	Inhibition of photosynthesis at Photosystem II	C3/22
Targa Super	Quizalofop-P-ethyl	Aryloxyphenoxy-Propionate 'FOPs'	Inhibition of acetyl CoA carboxylase (ACCase)	A/1
Boxer	Prosulfocarb	Thioicarbamate	Inhibition of lipid synthesis- not ACCase inhibition	N/8
Oratio	Carfentrazone-ethyl	Triazolinone	Inhibition of protoporphyrinogen oxidase (PPO)	E/14
Cato	Rimsulfuron	Sulfonylurea	Inhibition of acetolactate synthase ALS (acetohydroxy acid synthase AHAS)	B/2
Bandur	Aclonifen	Diphenylether	Inhibition of carotenoid biosynthesis (unknown target)	F3/13
Cirrus 50 WP	Clomazone	Isoxazolidinone	Inhibition of carotenoid biosynthesis (unknown target)	F3/13

Table 2: Herbicidal treatments used in the experiment

Trade Name	Active Ingredient	Dose (L ha^{-1})
Control (Mechanical weed control)		
Oratio	Carfentrazone	0.03
Kontakt 320 SC	Phenmedipham	1.50
Lentagran WP	Pyridate	1.00
Targa Super	Quizalofop-P	2.00
Boxer	Prosulfocarb	5.00
Cato	Rimsulfuron	0.03
Bandur	Aclonifen	3.01
Cirrus 50 WP	Clomazone	0.30

Table 3: Effect of herbicide application on leaf yield (t ha^{-1}) of artichoke

Herbicidal Treatments	First Harvest	Second Harvest
Control (Mechanical weed control)	28.73 a	56.05 a
Carfentrazone	28.46 a	50.03 ab
Phenmedipham	27.33 a	44.73 bc
Pyridate	27.80 a	38.41 cd
Quizalofop-P	27.01 a	52.26 ab
Prosulfocarb	28.58 a	51.93 ab
Rimsulfuron	29.10 a	33.90 d
Aclonifen	19.60 b	31.20 d
Clomazone	27.58 a	31.70 d
P value	0.006	< 0.001
LSD α 5%	*4.43*	*9.86*

Results

Herbicides applied to artichoke during both phases of artichoke growth affected the leaf yield significantly (Table 3). After the first harvest, minimum leaf yield was obtained where Aclonifen was applied after the germination of artichoke plants. Aclonifen influenced leaf yield was statistically different from the respective values of leaf yields in response to all other herbicidal treatments (incl. control). After first harvest no differences was observed between the herbicide treatments (exclusive Aclonifen) in comparison with the control (Table 3). After second leaf harvest, maximum leaf yield was obtained in control followed by the application of Quizalofop-P and Prosulfocarb (Table 3); whereas low leaf yields were harvested from the application of Aclonifen, Clomazone, Rimsulfuron and Pyridate (Table 3).

During the first growth phase of artichoke significant effect of herbicides on quantum yield of chlorophyll fluorescence was observed (Fig. 2). Herbicide Pyridate produced an adverse effect on the leaves of artichoke just after its application, as visible by the quantum yield value one day after the herbicide application, which is statistically lower than all other treatments. All other experimental treatments at this time showed no difference to the yield of chlorophyll fluorescence than control. The data presented at one week after herbicide application showed that Aclonifen affected the crop most adversely followed by a comparatively low adverse effect of Clomazone and Pyridate. Artichoke crop recovered against the adverse effects of Pyridate and Clomazone as visible by the chlorophyll fluorescence yield of the leaves obtained at two weeks after herbicide application. Although the intensity of the adversity of Aclonifen was reduced at this time, even then it was found to be statistically lower than that of all other treatments. Chlorophyll fluorescence data recorded at three weeks after herbicide application showed a mixed response to the applied herbicides. The data recorded at four weeks after herbicide application show a non-significant response of the chlorophyll fluorescence yield value to the herbicides used giving a clue about the recovery of the crop against the adverse effects of the herbicides.

Maximum quantum yield of chlorophyll fluorescence at one day after herbicide application during second growth phase 2008 was obtained by the application of Rimsulfuron, which was slightly higher when compared with that of control (see Fig. 3). Minimum yield value of chlorophyll fluorescence was obtained by the application of Aclonifen that was almost similar with that of all experimental treatments with the exception of Rimsulfuron, Clomazone, Haloxyfop and Phenmedipham. At one week after herbicide application quantum yield of chlorophyll fluorescence obtained by the application of both Haloxyfop and Prosulfocarb was statistically similar with that of control, which was also significantly similar with that of Phenmedipham and Rimsulfuron. At three weeks after herbicide application minimum quantum yield was observed after the application of Aclonifen with a slightly higher one at the application of Clomazone and these two were significantly same with each other. Same was found for Pyridate and Phenmedipham, which show the same trend and which had statistically lower yield values in comparison with that of the herbicides showing most adverse

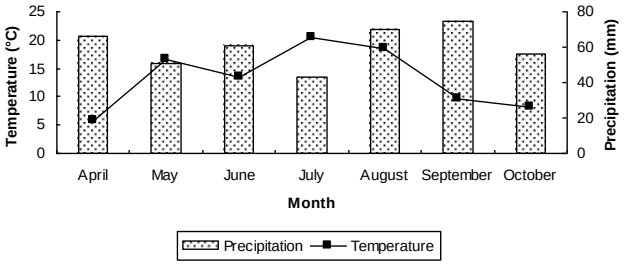

Fig. 1: Air temperature (°C) and precipitation (mm) data during the experimental period

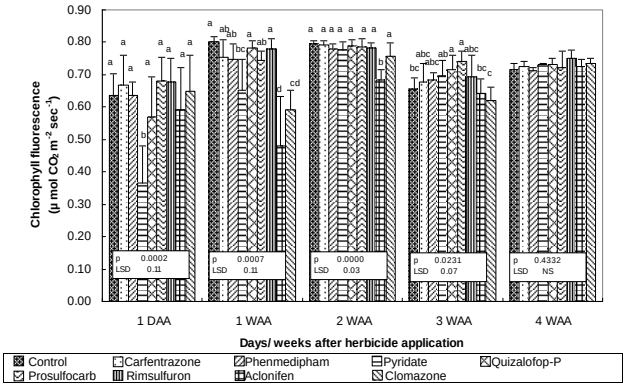

Fig. 2: Effect of herbicides on photosynthetic yield (µmol CO_2 m^{-2} s^{-1}) of artichoke leaves under light adapted conditions in Giessen during 1st growth phase

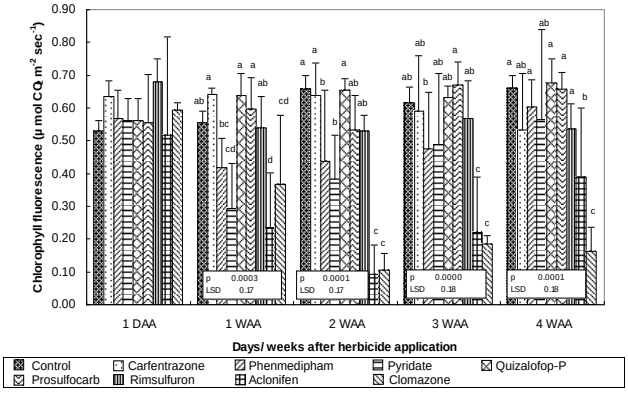

Fig. 3: Effect of herbicides on photosynthetic yield (µmol CO_2 m^{-2} s^{-1}) of artichoke leaves under light adapted conditions in Giessen during 2nd growth phase

effects. This trend of the response of chlorophyll fluorescence continued with a very little variation till four weeks after herbicide application, where data were recorded for the last time but the quantum yield started increasing for the herbicides that show an adverse effect and keep getting closer to that of the value obtained at control plots. This shows that the crop was not able to recover against the toxic effect imposed by a few of herbicides particularly that of Clomazone and Aclonifen, which showed statistically lower values of quantum yield when compared with that of control at four weeks after herbicide application. At this time these two herbicides showed statistically different quantum yields between one another too.

Chlorophyll fluorescence data and the calculated quantum yields recorded under dark adapted conditions also showed the same trend as that of measured under direct sunlight conditions and for the reason are not presented.

Discussion

Minimal leaf yield was found in the treatment where Aclonifen was applied as post emergence herbicide and was statistically lower than that of all other experimental treatments including control. Adverse effect of Aclonifen may be related to the mode of action of the herbicide, which inhibits carotenoid biosynthesis although the target is unknown (HRAC, 2013). This finding confirms the adverse effect imposed by the mentioned herbicide and artichoke was not able to recover fully against the stress. Both herbicides Clomazone and Aclonifen inhibit the biosynthesis of carotenoids and are selective in nature (HRAC, 2013). That is the reason why they kill only targeted weeds but additional they can induce stress in the crop. Maximum leaf yield obtained in control confirms the idea that all the used herbicides suppressed the growth of artichoke, which was not able to recover against this stress fully till the end of the growing season. Maximum leaf yield in control may be a result of favorable environmental conditions of improvement in soil structure, aeration and artificial mulch and to the early advantage of this treatment as it got no stress in the form of chemicals applied after germination. Low leaf yield obtained by the application of Pyridate during second harvest supports the finding that this herbicide had adverse effect on the artichoke leaves. The adverse effect of Pyridate and Phenmedipham may be related to the mode of action of these herbicides which inhibit photosynthesis at photosystem II (Table 1). The difference in the adverse effect of these herbicides may be due to different chemical groups of both compounds as Phenmedipham belongs to the group of 'Phenyl-carbamate' and Pyridate belongs to 'Phenyl-pyridazine' (HRAC, 2013). Both herbicides inhibited the photosystem for a specific period and thus remained behind in the accumulation of photosynthates and as a result produced lesser leaf yield when compared with other experimental treatments. Comparison of both growth phases of artichoke showed that leaf yield obtained in second growth phase of artichoke was higher than that of first growth phase. It may be related to the different environmental conditions prevailing during these growth phases. Both growth phases of artichoke differed in their length in addition to precipitation and air temperature, where first growth phase of artichoke prolonged to around 109 days in comparison to that of 71 days for the second growth phase. Average air temperature during the first growth phase

was found to be 20.2°C, which was almost double than that of the second growth phase i.e., 10.4°C.

Chlorophyll fluorescence has been used to provide a prompt, nondestructive analytical method for detection and quantification of damage to the leaf photosynthetic apparatus in response to environmental stress (Palta, 1992; Percival, 2005). Changes in chlorophyll *a* fluorescence due to altered photosystem II activity caused directly or indirectly by stress are measured in this technique. Krause and Weis (1991) and Schreiber *et al.* (1994) reported chlorophyll fluorescence analysis as sensitive and early indicator of damage to photosynthetic apparatus. Chlorophyll fluorescence can provide insight to the ability of a plant to tolerate environmental stresses and the extent to which these stresses have damaged the photosynthetic apparatus and advent and refinement of portable fluorometers have made the measurements possible under field conditions (Maxwell and Johnson, 2000). Refinement of fluorescence techniques and dark adapted measurements made in combination with that of light adapted measurements allow the extremely detailed analysis of the photosynthetic performance under field conditions (Maxwell and Johnson, 2000).

Methy *et al.* (1994) reported the ability of chlorophyll fluorescence measurements to detect frost and heat stress in plants, where heat stress was detected by light induced chlorophyll fluorescence whereas Rfd (fluorescence decrease ratio) values showed a decrease in response to the increasing temperature. Miyazawa and Yahata (2006) compared photosynthetic carbon assimilation rate and at the same time, recorded ETR through photosystem II, under field conditions. The authors concluded that ETR increased by increase in leaf temperature until peak values were attained. The authors also reported that light saturated rate of photosynthesis reached its maximum level at lower leaf temperature and decreased with increasing leaf temperature, as the specific factor of Rubisco to CO_2 decreased with decreasing temperature.

Gaillardon *et al.* (1989) reported that 90% of the absorbed Pyridate remained in the leaves of main crop and the target weeds, whereas 10% was transported mainly to shoot and a very minute amount to the roots. The authors also reported the higher susceptibility of younger weeds than that of older ones but, even though it was not correlated with the foliar absorption as the target weed in the study absorbed less Pyridate than that of the main crop. Pyridate does not absorb light at wavelengths higher than 290 nm, is rapidly hydrolyzed to CL 9673 even in air dried soil. CL 9673 is further degraded and CO_2 and several minor products and soil-bound residues are formed. The primary transformation product of Pyridate is CL 9673 (6-chloro-3-phenyl-4-hydroxy-pyridazine). Pyridate is basically the carrier form while CL 9673 is the physiologically active ingredient. Pyridate was shown to be predominately transformed by chemical hydrolysis to CL 9673. The data indicated that soil transformation was relatively rapid, even under conditions of low soil moisture, and consequently Pyridate was considered to be of little environmental concern. CL 9673 was shown to be primarily transformed by biological processes. Under normal agricultural conditions CL 9673 would be biotransformed by the end of the growing season; however test results indicated that under conditions of very low rainfall, residues of CL 9673 may carry over to the next year. CL 9673 was shown to be highly soluble in water at pH 7, and therefore, would be expected to leach readily in soils of neutral to alkaline pH. When comparing the exposure expected under field situations to levels causing acute toxicity, the acute risk to birds and wild mammals, from the use of Pyridate, was considered to be low (Anonymous, 1991).

Aclonifen and Clomazone inhibit isoprenoid biosynthesis at the level of isopentylpyrophosphate, at the very start of the pathway for carotenoid biosynthesis. This effect is not specific to the production of carotenoids, but the mode of action results from photodynamic damage due to the inhibition of carotenoid biosynthesis. Photodynamic damage is cellular harm caused by absorption of light energy by a molecule unable to safely dissipate the energy. This results in the lack of pigmentation in the leaves and the inhibitor blocks production of carotenoids. Chlorophyll molecules in the absence of carotenoids are more susceptible to bleaching in sunlight. No or low pigmentation in plants results in low photosynthetic rate and as a result plants will die once reserves of energy in the seed are depleted (Dayan and Duke, 2003). Artichoke crop recovered against the stress imposed by the post emergence application of both these herbicides possibly by the emergence of new leaves and biological decomposition of the chemical. Although it showed recovery against the adverse effects as shown by the quantum yield measured at four weeks after herbicide application but it was not complete recovery as is visible in lower biomass production of artichoke leaves in both the treatments.

Field (1983) and Hirose and Werger (1987) concluded that photosynthetic attributes vary among the leaves of different species, age of the leaves and the environmental light. The authors also reported that in order to attain maximum carbon gain by the use of limited resources arrange the leaves within a crown with high photosynthetic activity and the ones with low photosynthetic activity under shade. Quantum yield of artichoke leaves detected under direct sunlight conditions during the experimental year showed that Pyridate affected photosynthesis II right after its application (one day after herbicide application). This effect went on increasing and was followed by adverse effects of Aclonifen and Rimsulfuron, which showed their effect at one week after herbicide application. The adverse effect of all herbicides on chlorophyll fluorescence started decreasing from three weeks after herbicide application and it was near to normal (in comparison with that of control) at four weeks after herbicide application. Although these herbicides do not affect the photosynthesis directly, but the

stress imposed by these caused discoloration of the leaves and as a result chlorophyll fluorescence and electron transport rate were affected to a certain extent. Pyridate showed the adverse effect on chlorophyll fluorescence measured under direct dark adapted conditions at one day after herbicide application. Aclonifen showed the adverse effect at one week after herbicide application followed by that of Clomazone and Pyridate and this stress was recovered by artichoke through two weeks after herbicide application towards four weeks after herbicide application. Failure of artichoke for complete recovery against the herbicide stress during second growth phase, 2008 may be a reason of the environmental factors like sunlight and air temperature. Artichoke flourishes well under bright and sunny days, which prevailed during the first growth phase of artichoke growth and the crop recovered against this stress. Contrarily, less sunshine and lower air temperature were observed during the second growth phase of artichoke in 2008. These provided unfavorable conditions for crop growth and as a result artichoke could not recover completely against the stress.

In conclusion, different groups of herbicides impose a stress on the non-target plants (artichoke in the study) that differs in its intensity. The study leads to the idea that artichoke can recover against the herbicide stress depending on the severity of the stress in different times, but influences the biomass production of the crop. Herbicide stress can be identified through chlorophyll fluorescence measurement.

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