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Effects of post-emergence weedicides on yield, polyphenolic acids, flavonoids and antioxidant potential of artichoke leaves

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Artichoke is well known for its various medicinal properties i.e., lipid-lowering and antioxidant activity. Antioxidant activity of artichoke is mainly caused by the presence of various biologically active compounds, especially polyphenols. These polyphenols are being affected by a variety of environmental and cultivation factors including the application weedicides. The current study was aimed at clarifying the effects of various commonly used post-emergence weedicides (Phenmedipham, Pyridate, Quizalofop-p-ethyl, Prosulfocarb, Carfentrazone-ethyl, Rimsulfuron, Aclonifen, and Clomazone) on the production of polyphenolic compounds, including the flavonoids and caffeoylquinic acids. Weedicides were applied during the cultivation of artichoke in two growth phases. Polyphenols were analyzed in leaf extracts of artichoke using the HPLC method. In addition, the antioxidant activity of the leaf samples was determined. Results revealed minimum production of dry matter in the treatment with aclonifen (2.61 t ha^{-1}). While different weedicide showed variable effects after each week (1^{st}, 2^{nd}, 3^{rd}, 4^{th}) of application. Significant difference among the various treatments of weedicide during both growth phases *i.e.,* concentration of polyphenolics is being remarkably affecting by treatments with various weedicide. A positive correlation CQA (caffeoylquinic acid) with flavonoids on one hand and with ORAC (Oxygen Radical Absorbance Capacity) on the other showed the synergism among these. Moreover, the antioxidant capacity of artichoke leaf extracts is influenced by the content of caffeoylquinic acids and flavonoid in addition to some other antioxidants (carotenoids, terpenoids etc.). Weedicide application can lead to a reduction in phenolic constituents of artichoke leaf samples; however, this negative impact is quickly compensated during further growth and development of plant under moderate climate conditions.
Keywords: Artichoke, Weedicide, Caffeoylquinic acids, Flavonoids, ORAC.

INTRODUCTION

Cynara cardunculus L., commonly known as artichoke, is a cross pollinated and heterozygous plant belonging to the Asteraceae family. Artichoke can be divided into two sub-groups based on phenotypic characters i.e., the synchronized eatable portion known as cardoon and the leafy enveloped portion known as globe artichoke (Hanelt *et al.*, 2001, Frutos *et al.*, 2019).

Artichoke containing edible parts are widely consumed in food products as a main course meal as well as salad (Behara and Pharm 2011). Production of artichoke is credited to Italy as the world's largest producer of artichoke while in central and south-east European countries, it is also well produced (FAOSTAT, 2021). Artichoke is being used in medicines since the 1970s and its leaves have been proved with distinguished medicinal benefits including the reduction in blood cholesterol level. Medicinal properties of artichoke are normally related to the presence of polyphenolic compounds, which act as antioxidants, scavenging the free radicals in blood veins. Polyphenolic compounds or polyphenols are a large group of compounds, primarily consisting of two major sub-groups as phenolic acids and flavonoids (Seabra *et al.*, 2002, Fratianni *et al.*, 2007, Ali and Neda 2011).

Leaf extracts of artichoke have been reported with a comparable high content of polyphenols, consisting of 2 – 4 % of total phenolic acids especially chlorogenic acid, cynarin and caffeic acid, 3 – 4 % sesquiterpene lactones and up to 1%

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total flavonoids particularly scolymoside, cymaroside and luteolin (Koubaa *et al.*, 1999, Joy and Haber 2007). Leaf extracts also contain various other bioactive substances i.e., phytosterols, tannins and inulin (Alamgir 2017).

A major problem in the cultivation of artichoke is that the plant stands become heavily weedy due to the slow juvenile development and the larger row spacing (Monaco *et al.*, 2002). To control these weeds, various types of weedicides are being used, and it is the most common practice to combat this problem. These weedicides are applied either in pre-emergence or in post emergence. Although the application of weedicide improves the growing conditions of plant stands, they can also trigger adverse effects if applied inappropriately i.e., inhibiting photosynthesis by degrading the chlorophyll. It may also badly affect the fluorescence ability of leaves and various vital physiological compounds i.e., polyphenolic compounds (Miyazawa and Yahata 2006).

Polyphenolic compounds are secondary metabolites of plant physiological processes, but they can be also involved in regulating the metabolism, increasing immunity, and acting as antioxidants (Tuladhar *et al.*, 2021). Polyphenolic compounds are categorized into two major groups as phenolic acids and flavonoids. Various phenolic acids including 3-, 4-, and 5-caffeoylquininc acids, caffeic acid, 1,3-dicaffeoylquinic acids (cynarin), 3,4-, 3,5-, 1,5-, and 4,5-dicaffeoylquinic acids, luteolin 7-glucoside; apigenin 7-glucoside and luteolin, are found in artichoke (Slanina *et al.*, 1993, Grancai *et al.*, 1994, Lattanzio 2013).

The current study was conducted to clarify whether the application of weedicide in artichokes for foliar use has an influence on the contents of phenolic acids and flavonoids. Furthermore, it was to be investigated whether possible changes in the polyphenol contents also alter the antioxidant capacity of the leaf extracts of artichoke as well as the interrelationships between the components investigated.

MATERIALS AND METHODS

***Experimental Design*:** The field experiment was conducted at the experimental station of the Institute of Agronomy and Plant Breeding I, University of Giessen (50° 36´ N & 8° 39´ with 158 m altitude) on a soil classified as Gleyic Fluvisol (IUSS Working Group WRB, 2015). The soil profile (0-100 cm) consisted of 36-48% clay and 45-58% silt with a humus content in the topsoil (0-30 cm) of 1.5-2.0%. Nitrogen fertilization (in total 80 kg ha^{-1}) was applied as basal dose at the time of seedbed preparation, whereas 20 Kg N ha^{-1} on 13th August 2008 after the first harvest. Long year average annual temperature was recorded as 13.19˚C and average precipitation as 435.06 mm $annum^{-1}$. Climatic data of the experimental station during the cultivation of artichoke (from April to October) is briefly expressed in Fig. 1.

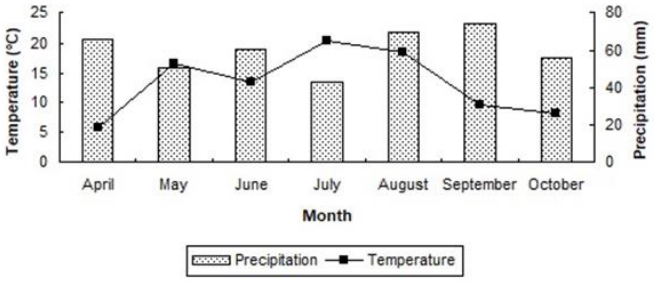

Figure 1. Climatic conditions at the experimental station Giessen, mean air temperature (°C) and precipitation sum per month (mm) data during the growth period of artichoke from April to October 2008

A randomized complete block design (RCBD) was used to establish the experimental model with four replications. Experimental plot size was maintained as 3.0 × 7.0 m, while the net plot was calculated to be 1.5 × 7.0 m. Intra row distance was maintained about 25 cm while distance between rows was 75 cm. Gobbo di Nizza cultivar of artichoke (commonly known as cardoon) was planted manually in approximately 2.5 cm soil depth on April 28, 2008, harvested on 7th August, 2008 for the first cut and on 6th November, 2008 for the second one. In total eight post emergence weedicide representing different active ingredients and modes of action were considered (Table 1) to analyze their effects on the polyphenolic compounds and on antioxidant potential in artichoke leaves.

***Study Parameters*:**

***Leaf Yield* :** Leaf yields of artichoke plants in t DM/ha were calculated by the method of Honermeier *et al*.,, (Honermeier *et al.*, 2009). Briefly, artichoke leaves were harvested from plants grown in middle rows of each plot and their fresh weights (g) were measured (W_1). Then leaves were dried in a hot air oven (105 ℃ till their constant weight) and dry weights were detected (W_2). The percent dry matter of the leaves harvested was calculated using equation given below, and their means were obtained and considered for true quantification.

$$Dry\ Matter\ (\%) = \left(\frac{W_2}{W_1}\right) \times 100$$

Dry matter was converted to tons per hectare by (t ha^{-1}) manipulation of data using following equation:

$$Dry\ Matter(\ t\ ha^{-1}) = fresh\ leaf\ yield\ (t\ ha^{-1}) \times dry\ matter\ (\%)$$

The laboratory analyses of the artichoke leaves (ORAC, CQA and flavonoids) were carried out based on leaf sample taken in weekly intervals *i.e.*, one week after application (1 WAA), two weeks after application (2 WAA), three weeks after application (3 WAA) and four weeks after application (4 WAA).

Table 1. Weedicide used in current study containing active ingredients and dose applied to artichoke

Sr.	Weedicide (active ingredient)	Chemical Family	Mode of Action	Dose (L ha^{-1})
1	Control	-	-	-
2	Carfentrazone-ethyl	Triazoline	PPO	5.00
3	Phenmedipham	Phenylcarbamate	Photosystem II	0.03
4	Pyridate	Pyridazine	Photosystem II	1.50
5	Quizalofop-p-ethyl	Aryloxyphenoxypropionate	ACCase	1.00
6	Prosulfocarb	Sulfonylurea	ALS	2.00
7	Rimsulfuron	Sulfonylurea	ALS	0.03
8	Aclonifen	Diphenylether	PPO	3.00
9	Clomazone	Isoxazolidinone	DOXP	0.30

Control: weed-free plot (no weedicide was applied, weeds were removed mechanically), ACCase: acetyl CoA carboxylase, ALS: acetolactate synthase, PPO: Photoporphyrinogen oxidase, DOXP: 1-Deoxy-D-xylulose 5-phosphate

***Analyses of Polyphenolic Compounds*:** Total flavonoids and total caffeoylquinic acids were analyzed and quantified using the High-Performance Liquid Chromatography (HPLC) oriented method described by Brand and Weschta (1991) with some necessary modifications described below. Chlorogenic acids and its isomers were quantified as caffeoylquinic acids and luteolin-7-glucoside (cynaroside) and its isomers were quantified as flavonoids in µg mL^{-1}.

Sample extraction: Artichoke leaf samples were oven dried at low temperature (40 ℃) till constant weight obtained and 50 mg of fine powdered material was subjected for extraction of sample to be analyzed for phenolic acids and flavonoids by the method described earlier in Ali (2018) with some further modifications. Briefly, samples for assay were extracted in methanol (80%) by sonication (30 min at 10 ℃) and centrifugation (6500 rpm, 20 min, 4℃). Supernatant was procured and micro-filtered (0.45 µm pore size) and preserved at – 20 ℃ until further analyses.

***Instrument and reagents*:** HLPC (Knauer Germany) used consists of a diode array detector (Model K-271) with a detector lamp (K02700). Further on, the device contained a degasser [K- 5004 (A 2015)], solvent organizer [K- 1500 (A 4012)], maxi star pump [K- 1001/VA (A 40301)], dynamic mixing chamber (A 0285) and marathon auto sampler [K-3800 (A 0759)]. These parts were purchased from 'Knauer', while column thermostat (Techlab K-7) was purchased from 'Techlab'. Pre column (SecurityGuard cartridges C 18 4 x 3.0 mm) and column (Synergi 4 µm Hydro-RP 80A 250 x 4.6 mm) were used from the company 'Phenomenex' and the software to run the system was from 'Knauer'. For the early part of the HPLC analysis stainless steel capillaries (outer diameter 1/16" and inner diameter 0.8 – 1.0 mm) along with 1/16"stainless steel ferrules, 1/16" stainless steel fittings and 1/16" stainless steel joints were used. These stainless-steel parts were then changed with plastic parts and the PEEK capillaries (inner diameter 0.17") and PEEK finger tight fittings were used for the analyses. Ratiolab injection filter 13 mm (0.45 µm) from the company 'Carl ROTH' was used to filter the samples from the test tubes to the injection vials. Eluent was containing phosphoric acid (0.5%), acetonitrile and water. Reagents were HPLC grade and purchased from Merck.

***Analysis methods*:** Mixture of eluent was prepared by gradient method i.e., all three solvents were mixed by automated function during the analyses. The wavelength of the detector was adjusted to 330 nm, while flow rate was adjusted to 1.0 mL min^{-1} and total assay time for one sample was 50 min. Peaks was observed after 5 min and before 33 min and considered for quantification and statistical analyses. Purified standard substances of chlorogenic acid (CGA), caffeic acid (CAF), cynarin (CYN) (for caffeoylquinic acids) were used to form a standard curve and to calibrate the system, while Luteolin-7-glucoside (L7G) was used as calibration standard for flavonoids. CGA, CAF and CYN were dissolved and diluted in methanol: water (40:60 v/v), while L7G was prepared in absolute methanol. Various concentrations of standards were used to calibrate the system based on ability of detection ability of HPLC system and correction factor was calculated according to the following equation:

$$Correction\ factor = \frac{Detected\ concentration}{Actual\ concentration}$$

***Quantification of polyphenols*:** Obtained concentrations of polyphenolic compounds were finally converted to percent of dry matter using the following equation:

$$\text{Concentration (\% DM)} = \left[\text{conc.}\left(\mu\frac{g}{mL}\right) \times 25\,mL \times 100\right] / [50mg \times 1000]$$

In above mentioned concentration (µg mL^{-1}) denotes the actual concentration in µg mL^{-1}, 25 mL volume was used for extraction, 50 mg leaf sample was used for extraction. While 1000 means the conversion of µg to mg and multiplication by 100 was done to calculate percentage.

***Antioxidant Activity*:** Antioxidant activity of polyphenolic compounds was determined by oxygen radical absorbance capacity (ORAC) assay, using the standard protocol adopted from Ou *et al.,* (2001) with certain modifications. A Fluoroskan Ascent FL plate reader (Thermo Scientific, Vantan, Finland) equipped with white 96-well microplates (Ratiolab, Dreieich, Germany) was used for this assay. The

assay was optimized by formation of a standard curve of trolox of known concentrations (75 and 150 µM). Analyses were conducted in phosphate buffer at pH 7.4 and 37 °C, peroxyl radicals were generated using 2, 2'-azobis (2-amidino-propane) dihydrochloride using the fluorescein as substrate. Fluorescence conditions were maintained as excitation at 485 nm and emission at 535 nm. Reaction cocktail in each well contained 150 µL of fluorescein (15nM) and 25 µL of sample/blank (phosphate buffer) or trolox standard (75 µM -150 µM). The plate was incubated for 30 minutes at 37 °C in the Fluoroskan Ascent followed by the addition of 25 µL of AAPH (final concentration 19mM) in each well to initiate the reaction. Microplates were immediately placed in the Fluoroskan Ascent FL and the fluorescence was monitored for 90 min at the interval of every 2 min. The results were obtained by calculating the area under the fluorescence curve and expressed as µM TE g^{-1} (micromoles of Trolox equivalents per gram) dry weight.

***Statistical analysis*:** Data obtained were subjected to Statistix 8.1 for ANOVA, regression and co-relation analyses. Probability levels (5%) were considered to analyze the difference among treatment means. Least significant difference (LSD) test was applied ($p \leq 0.05$) in the same statistical package. MS Excel 2010 was used for the calculation of standard deviations and preparation of graphs.

RESULTS

Leaf dry matter (t ha^{-1}) presented graphically in Figure 2 illustrates that maximum leaf dry matter yield was produced in the plots treated with rimsulfuron (4.02 t DM ha^{-1}), while minimum leaf yield was found in the variant applied with aclonifen (2.60 t DM ha^{-1}). Statistically, the level of DM leaf yields was significantly same in all treatments (including control) except the variant with aclonifen which has caused a clear reduction in leaf yield During the first phase of artichoke growth.

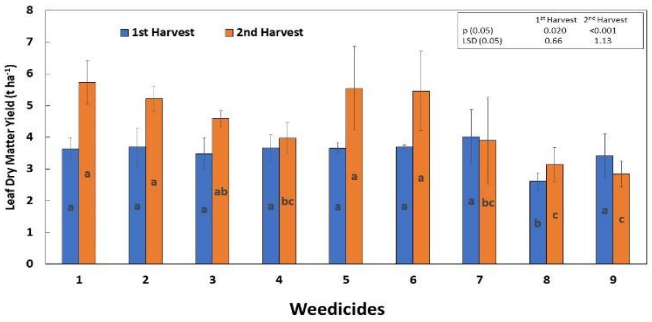

Figure 2. Effect of weedicide on leaf dry matter yield (t DM ha^{-1}) of artichoke in the field experiment Giessen, in both growth phases of artichoke.

Y-axis represents the production of leaf dry matter yield (t ha^{-1}), while X-axis indicates the treatments (1: Control, 2: Carfentrazone-ethyl, 3: Phenmedipham 4: Pyridate, 5: Quizalofop-p-ethyl, 6: Prosulfocarb, 7: Rimsulfuron, 8: Aclonifen, 9: Clomazone.

During the second growth phase leaf dry matter yield of artichoke plants was also significantly affected by the application of various weedicides (Figure 2). Maximum leaf yield (5.73 t DM ha^{-1}) was observed in the control (without weedicide) which was statistically identical with the yield in weedicide application of quizalofop-p-ethyl, prosulfocarb and carfentrazone. Visible lower dry matter yield level was found in the variants of pyridate, rimsulfuron, aclonifen and clomazone. The latter two had the lowest leaf dry matter yield of all variants which suggests an inhibitory effect of the weedicide ingredients contained therein (Figure 2).

A close observation of the data regarding first growth phase of the artichoke plants one week after weedicide application, the levels of CQA in the leaves were still very low (< 0.5%) (Fig. 3). CQA levels were then significantly higher in 2 WAA and 3 WAA (> 3%), while they decreased again somewhat in 4 WAA. A comparable trend, but at a lower level, was also observed for flavonoids.

The data regarding the antioxidant activity (ORAC) of the leaf extracts during the first growth phase arranged in figure 3a reveals that the values increased continuously and very strongly from the first date (1 WAA) to the fourth date (4 WAA) (Fig. 3a). Significant differences between the weedicide variants could only be found at 3 WAA. At this time, all weedicide, except carfentrazone, led to a statistically significant reduction in ORAC values. Furthermore, it can be seen that pyridate, compared to the other variants, caused the lowest antioxidant activity at all dates.

During the second growth phase, the level of ORAC values was lower than during the first growth phase and tended to decrease slightly from the first to the fourth date. Comparing the variants, only minor differences were observed. Overall, pyridate produced the lowest values (lower than the control) at all dates, while rimsulfuron achieved statistically significantly higher ORAC values at 2 WAA (Figure 3b).

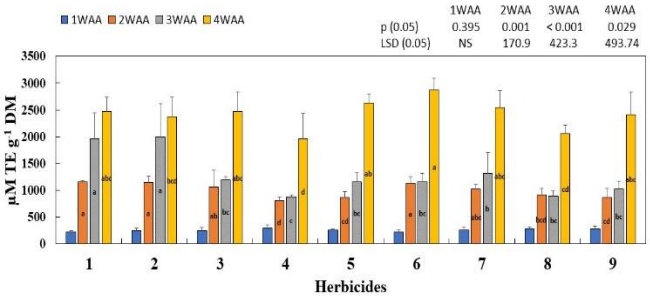

Figure 3a. Influence of various weedicide on the antioxidant activity (ORAC) in µM TE g^{-1} (micromoles of Trolox equivalents per gram of DM) of 1st growth phase of artichoke.

Y-axis represents the antioxidant activity (ORAC) in µM TE g^{-1} (micromoles of Trolox equivalents per gram of DM), while X-axis indicates the treatments (1: Control, 2: Carfentrazone-ethyl, 3: Phenmedipham 4: Pyridate, 5: Quizalofop-p-ethyl, 6: Prosulfocarb, 7: Rimsulfuron, 8: Aclonifen, 9: Clomazone.

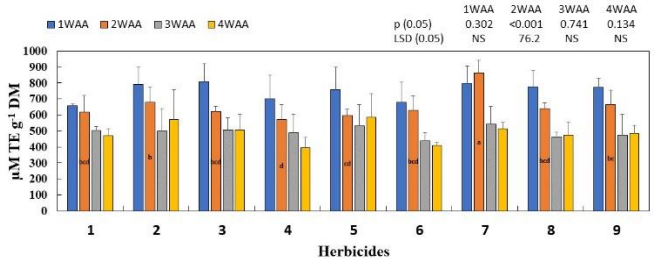

Figure 3b. Influence of various weedicide on the antioxidant activity (ORAC) in µM TE g^{-1} (micromoles of Trolox equivalents per gram of DM) of 2nd growth phase of artichoke.

Y-axis represents the antioxidant activity (ORAC) in µM TE g^{-1} (micromoles of Trolox equivalents per gram of DM), while X-axis indicates the treatments (1: Control, 2: Carfentrazone-ethyl, 3: Phenmedipham 4: Pyridate, 5: Quizalofop-p-ethyl, 6: Prosulfocarb, 7: Rimsulfuron, 8: Aclonifen, 9: Clomazone.

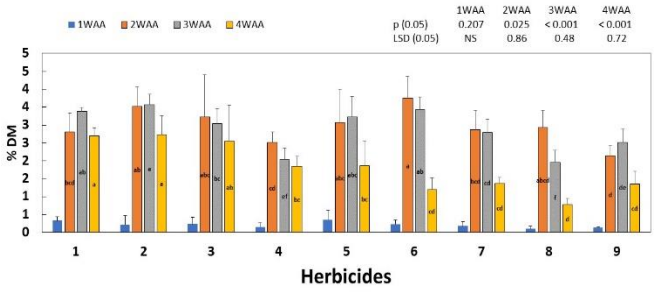

Figure 4a. Influence of various weedicide on the concentration of caffeoylquinic acids (CQA) in percent of leaves DM of 1st growth phase of artichoke.

Y-axis represents the caffeoylquinic acids (CQA) in percent of leaves DM, while X-axis indicates the treatments (1: Control, 2: Carfentrazone-ethyl, 3: Phenmedipham 4: Pyridate, 5: Quizalofop-p-ethyl, 6: Prosulfocarb, 7: Rimsulfuron, 8: Aclonifen, 9: Clomazone.

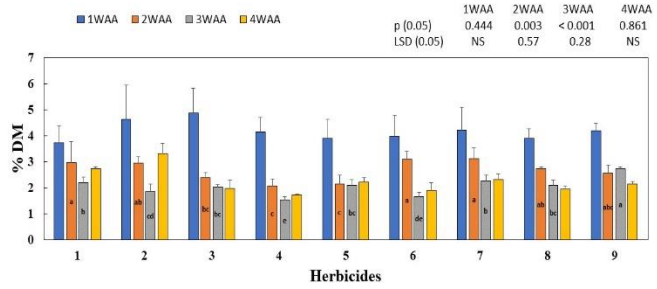

Figure 4b. Influence of various weedicide on the concentration of caffeoylquinic acids (CQA) in percent of leaves DM of 2nd growth phase of artichoke.

Y-axis represents the caffeoylquinic **acids** (CQA) in percent of leaves DM, while X-axis indicates the treatments (1: Control, 2: Carfentrazone-ethyl, 3: Phenmedipham 4: Pyridate, 5: Quizalofop-p-ethyl, 6: Prosulfocarb, 7: Rimsulfuron, 8: Aclonifen, 9: Clomazone.

The response of both phenolic groups, CQA and flavonoids were observed as non-significant after one week after application (1 WAA) and maximum content of CQA as well as flavonoids was found in the control (without weedicide). All weedicide treatments especially aclonifen and clomazone resulted in lower levels of polyphenols. However, the differences were not statistically significant.

Graphical presentation of the effect of the weedicide on the CQA content of the leaves after two (2 WAA), three (3 WAA) and four weeks (4 WAA) depicts clear differences in some cases, which increase with increasing time. Thus, at 2 WAA quizalofop-p-ethyl and clomazone caused the lowest (not statistically significant) and carfentrazone-ethyl the highest (statistically significant) levels of CQA. These differences increased after 3 WAA and 4 WAA, so that at 3 WAA significantly lower CQA levels were observed for three weedicide (quizalofop-p-ethyl, aclonifen, clomazone) compared to the control. After 4 WAA, the inhibitory effect extended to six weedicide (quizalofop-p-ethyl, prosulfocarp, carfentrazone-ethyl, rimsulfuron, aclonifen, clomazone). The reduction in CQA levels was particularly drastic for the latter four weedicide. The same trend as for CQA was observed for flavonoid content, but the differences were somewhat smaller (Fig. 4a & 5a).

In the second growth phase, the CQA and flavonoid contents of the artichoke leaves were slightly higher overall than in the first growth phase. The differences between the weedicide variants were also no longer as clear as in the first growth phase. However, it was also clear here that the contents decreased from 1 WAA to the later dates. Furthermore, phenmedipham did not have an inhibitory effect on any date. On the other hand, quizalofop-p-ethyl had a clearly negative effect on all dates and carfentrazone-ethyl on date 3 WAA. The effect of all other weedicide was rather indifferent (Figure 4b & 5b).

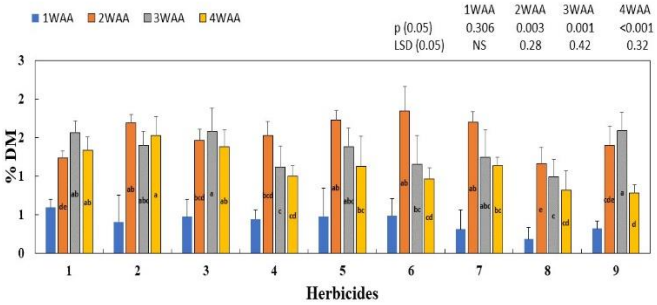

Figure 5a. Influence of various weedicide on the concentration of flavonoids in percent of leaves DM of 1st growth phase of artichoke.

Y-axis represents the concentration of flavonoids in percent of leaves DM, while X-axis indicates the treatments (1: Control, 2: Carfentrazone-ethyl, 3: Phenmedipham 4: Pyridate, 5: Quizalofop-p-ethyl, 6: Prosulfocarb, 7: Rimsulfuron, 8: Aclonifen, 9: Clomazone.

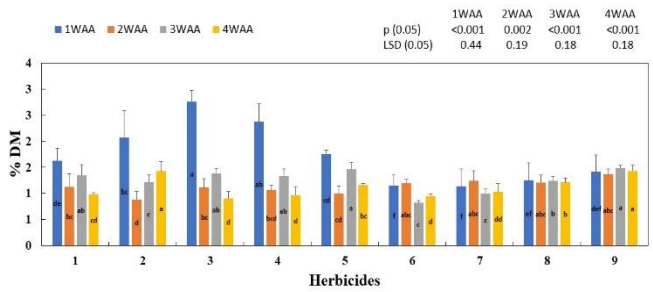

Figure 5b. Influence of various weedicide on the concentration of flavonoids in percent of leaves DM of 2nd growth phase of artichoke.

Y-axis represents the concentration of flavonoids in percent of leaves DM, while X-axis indicates the treatments (1: Control, 2: Carfentrazone-ethyl, 3: Phenmedipham 4: Pyridate, 5: Quizalofop-p-ethyl, 6: Prosulfocarb, 7: Rimsulfuron, 8: Aclonifen, 9: Clomazone.

The correlation between the chemical parameters (CQA, flavonoids, ORAC) during the first growth phase expressed graphically in figure 6a) depicts a positive correlation between CQA and flavonoids ($p \leq 0.05$). This positive correlation could be observed with r=0.770 (1 WAA), r=0.483 (2 WAA), r=0.426 (3 WAA) and r=0.755 (4 WAA) at all four dates. The correlation among the polyphenols (CQA and flavonoids) on the one hand and the antioxidant activity (ORAC) on the other hand was not clear. At 1 WAA, a strong negative correlation between CQA/flavonoids and ORAC was determined. On the other hand, at the subsequent dates (2 WAA, 3 WAA, 4 WAA) a slightly positive correlation was observed, which was statistically significant between ORAC and CQA only at 2 WAA and 3 WAA (Fig. 6a).

In the second growth phase, there was also a positive correlation between CQA and flavonoids (with correlation

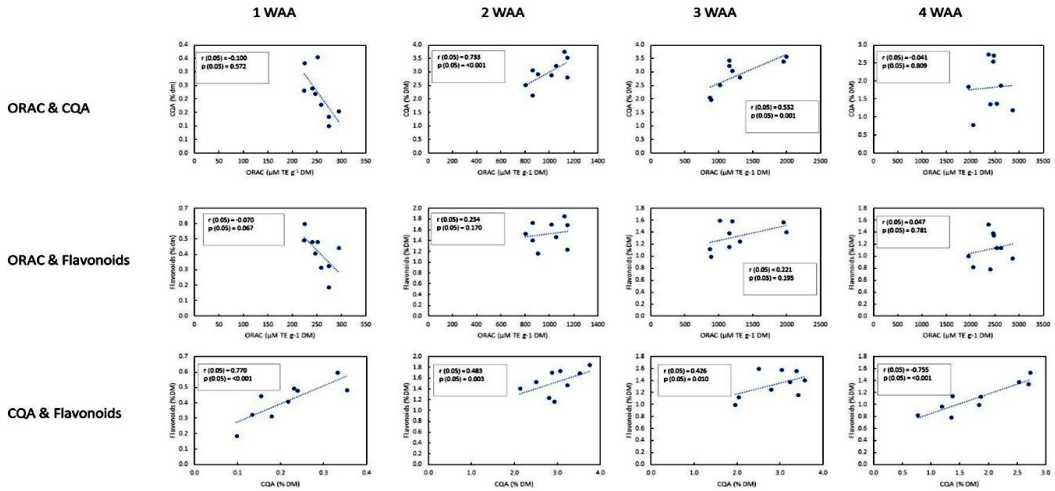

Figure 6(a). Correlation between ORAC, CQA and flavonoids in leaf extracts of artichoke, during the first growth phase, field experiment Giessen.

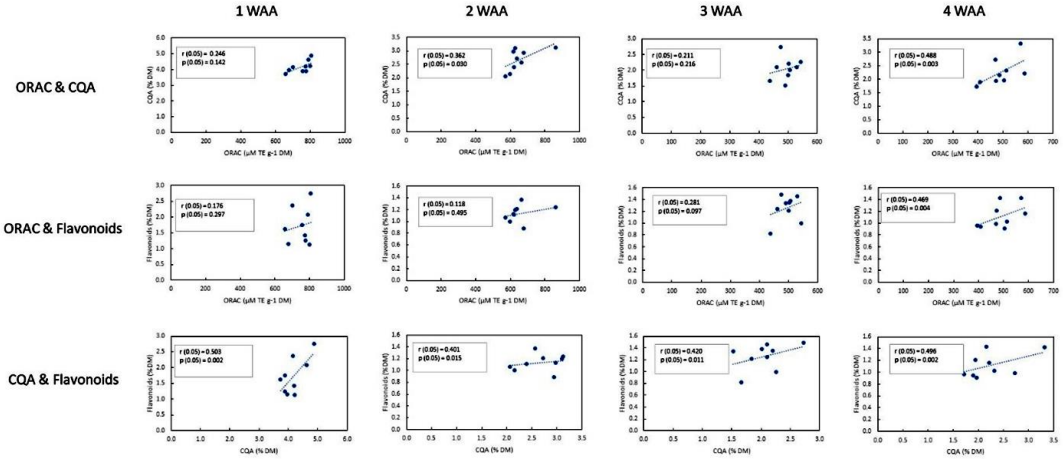

Figure 6(b). Correlation between ORAC, CQA and flavonoids in leaf extracts of artichoke during the second growth phase, field experiment Giessen.

coefficients ranging from r=0.401 to 0.503), which was statistically significant ($p \leq 0.05$) at all four dates (Fig. 6b). The correlation between ORAC and polyphenols (CQA, flavonoids) tended to be weakly positive at all dates. Only at two dates was this positive association statistically significant (between ORAC and CQA at 2 WAA and 4 WAA and between ORAC and flavonoids at 4 WAA) (Fig. 6b).

DISCUSSION

In the study conducted here, the artichoke was cultivated for foliar use to obtain leaf extracts, which took place twice a year. Optimal growth conditions are required to achieve high leaf yields and active ingredient contents (CQA, flavonoids). In addition to water and nutrient supply, this also includes avoiding weed competition to enable good leaf development. Besides mechanical weed control, mainly weedicides are used, which have a low phytotoxicity towards artichoke and should not affect the synthesis of polyphenols as active ingredients.

Looking first at the temporal dynamics of caffeoylquinic acids (CQA) and flavonoids over the four study dates during the first growth cycle, very low CQA (< 0.5%) and flavonoid (< 0.8%) contents were measured at 1 WAA, which already adjusted to a significantly higher level from 2 WAA and then stabilised. Two reasons are suspected of this effect. Firstly, the synthesis and accumulation of phenolic compounds in the leaves of the very young plants was not yet sufficiently developed, which improved significantly after two weeks. This assumption is supported above all by the fact that very low CQA levels (flavonoid levels were slightly higher) were also found in the control at this time.

Secondly, all weedicides apparently led to a very strong stress reaction of the still very young plants immediately after application (1 WAA), resulting in a reduction of CQA and flavonoid content. There is evidence in the literature for both negative and positive effects of weedicide application on phenolic compounds. For example, Alla and Younis (1995) observed in young plants that the weedicides trifluralin (in soybean) and fluometuron (in maize) reduced the enzyme activity of phenylalanine ammonia lyase (PAL) and chalcone isomerase (CL), two key enzymes of polyphenol biosynthesis, resulting in lower polyphenol (anthocyanin) contents. In other studies, however, positive effects of weedicides on PAL and CL, as well as on phenolic compound contents were also found (Scarponi *et al.,* 1992, Alla and Younis 1995, Subba Rao and Modhulety 2005, Pakdaman Sardrood and Mohammadi Goltapeh 2018).

In the second growth cycle, the CQA and flavonoid contents after 1 WAA were significantly higher than in the first growth cycle. The reasons for this can be seen on the one hand in the fact that the plants (roots and apex) are already well established and have a higher physiological age and therefore are less sensitive. On the other hand, the growth conditions (higher amount of UV light) at this time may also have contributed to higher polyphenol contents.

Looking at the effect of the individual weedicides, in the current study aclonifen was the only weedicide in the trial that led to reduced leaf yields in both growth cycles. The symptoms of aclonifen application were characterised by bleaching the leaves and reduced leaf biomass. At the same time, CQA and flavonoid contents of the leaves were reduced in the first growth cycle after 3 and 4 WAA. The observed effects are apparently the result of the physiological action of aclonifen. Up to now, two pathways were known for this: on the one hand, the inhibition of carotenoid biosynthesis (Ali and Honermeier 2013, Sparks and Nauen 2015) and, on the other hand, the inhibition of PPO (protoporphyrogen oxidase), which catalyses the final steps in the production of chlorophyll (Kilinc *et al.,* 2009). In addition, Kalau *et al.*, (Kahlau *et al.,* 2020) recently also identified solanesyl diphosphate synthase as a target and further mode of action of aclonifen.

The application rate of aclonifen with the dosage of 3 L ha^{-1} used in our trial corresponds to the official (state) recommendation for various medicinal and aromatic plants such as marigold and coriander. For other species such as mint, sage, and celery, however, only 1 L ha^{-1} (pre-emergence) is recommended. It is therefore suspected that with 3 L ha^{-1} a very high and phytotoxically effective amount was applied to artichokes which could also have triggered the phytotoxic reactions.

In the second growth phase, besides aclonifen, clomazone also had a negative influence on the leaf yield (e.g., in the second growth cycle) and the CQA concentration (at 3/4 WAA in the first growth cycle) of the artichoke. Clomazone inhibits the enzyme DOXP (1-deoxy-D-xyulose-5-phosphate synthase), a key component of isoprenoid synthesis, which in turn leads to disruption of chlorophyll and carotenoid synthesis (Habiba *et al.,* 1992, Scott *et al.,* 1994). Thus, despite different active ingredients, aclonifen and clomazone appear to have comparable effects on the plant (Sparks and Nauen 2015). The artichoke plants were not able to compensate for the stress caused by these two weedicides, which is why they are not recommended for use in artichokes at the dosage used here.

Negative effects on leaf growth (reduced yields in the second growth cycle) and leaf constituents (reduction of CQA in the first and second growth cycle) were also observed with pyridate. Pyridate inhibits photosynthesis by blocking electron transport through photosynthesis II (PS II). The weedicide is hydrolysed in plant cells to specific metabolites (e.g., the major metabolite CL9673), whose detoxification to conjugates is tolerated in some crops (e. g, maize, peanut) (Gimeenez-Espinosa *et al.,* 1995). It is suspected that the metabolites formed during the degradation of pyridate are not so well tolerated by the artichoke plant. Furthermore, there

seems to be a negative effect on the synthesis of polyphenols, which is related to the disruption of photosynthesis II.

The weedicide rimsulfuron was included in the studies as a representative of the sulfonyl-ureases, which have favourable environmental and toxicological properties (Beyer *et al.*, 1988). In the experiment conducted, however, it was shown that in the second growth cycle of the artichoke plants, reduced leaf yields occurred in the rimsulfurane variant. In contrast, no negative yield effects were observed in the first growth cycle, but a reduction in the CQA content at 3 and 4 WAA. For these reasons, it was assumed that the physiological stress triggered by rimsulfuron also had an unfavourable effect on the synthesis of phenolic compounds. This finding contrasts with pot experiments with maize and soybean, where rimsulfuron caused an improved activity of the enzymes PAL (phenylalanine ammonia lyase) and CL (chalcone isomerase) and thus an increase in hydroxy-phenolic compounds (Alla and Younis 1995).

Another weedicide (prosulfocarb) was tested which is characterised as a thiocarbamate weedicide to control grasses and works by interrupting the synthesis of long-chain fatty acids (Busi 2014). In the trial conducted, prosulfocarb was well tolerated by the artichoke plants (same leaf yields as in the control). This could be because prosulfocarb has a stronger effect on monocotyledonous plants (grasses) than on dicotyledonous plants (e.g., artichoke). However, prosulfocarb had a partial negative effect on the CQA or flavonoids of the artichoke leaves. Therefore, it must be assumed that the synthesis of phenolic compounds in the artichoke plant is inhibited by prosulfocarb.

Among all weedicide tested in the trial, four weedicides could be identified (namely prosulfocarb, carfentrazone-ethyl, phenmedipham and quizalofop-p-ethyl) that had no negative effects on artichoke leaf yield and were therefore well tolerated. Of these weedicides, only carfentrazone-ethyl and phenmedipham also had a positive effect on the CQA and flavonoid contents of artichoke leaves which had the same level as the control. Both weedicides have different modes of action. Phenmedifam inhibits the electron transport of PS II, which has been demonstrated by fluorescence measurements (Abbaspoor and Streibig 2007). The weedicide has a high lipophilicity and is concentrated in the biological membranes, which does not occur with other PS II inhibitors (Ravanel *et al.*, 1990). In contrast, the weedicide carfentrazone-ehtyl is a protox (protoporphyrinogen oxidase) inhibitor that triggers the enzymatic response to oxidative stress (increase in lipid peroxidase) and leads to degradation of chlorophyll and carotenoids (Langaro *et al.*, 2016). Both weedicides were sufficiently effective when applied to artichoke (because leaf yields were as high as in the control), caused fewer toxic effects and apparently did not affect the synthesis of phenolic compounds (CQA, flavonoids) in the leaves. Thus, these weedicides with their principles of action and under the experimental conditions presented here are assessed as very positive for application in artichokes for foliar use and CQA production.

Regardless of the influences of the weedicides and the developmental cycle of the plants on the polyphenol contents, the level of CQA and flavonoid contents in the artichoke leaves were quite high, also in comparison with previous research (Pandino *et al.*, 2015). CQA contents of more than 2 % and partly more than 3 % as well as very high flavonoid contents of more than 1 % indicate very favourable conditions (e. g., soil, climate and cultivar used) that existed during the trial.

Thus, regarding climatic conditions, it is known that moderate day-time temperatures of 20 °C and night-time temperatures of 15 °C are more favourable for the synthesis and accumulation of polyphenols than high air temperatures of 30 °C (day) resp. 20 °C (night) (Graham and Patterson 1982, Tomás-Barberán and Espín 2001). The average air temperature during the experimental study was between 18.1 to 25 °C, while during the cultivation period, precipitation was 179 mm for the first growth phase, while it was increased to 186 mm in the second growth cycle. These conditions are thought to have favoured the accumulation of polyphenols in the artichoke leaves (Munin and Edwards-Lévy 2011).

To determine the antioxidant capacity of the leaf extracts, the ORAC (oxygen radical antioxidant capacity) method, measuring the decrease in fluorescence of a molecular probe due to chemical damage triggered by free radicals, was used (Pandino *et al.*, 2015). This method is widely used among other methods to detect the antioxidant capacity in plants and plant-based foods (Scott *et al.*, 1994, Wang and Lin 2000, Ehlenfeldt and Prior 2001, Ou *et al.*, 2001).

The antioxidant capacity of the studied artichoke samples increased significantly in the first growth cycle from 1 WAA to 4 WAA, whereas they were constant and at an intermediate level in the second growth cycle. The increase in ORAC values in the first growth cycle corresponds to the increase in CQA and flavonoids from 1 WAA to 2 WAA. Therefore, it seems plausible that the ORAC values were dependent on the content of polyphenols in the leaves, as expected. In contrast, this correlation was not obvious at 4 WAA (first growth cycle), as very high ORAC values contrasted with decreasing CQA values. It should be noted that ORAC is not only influenced by CQA and flavonoids, but also by other components (e.g., carotenoids and terpenoids), which were not determined here. This is confirmed by the studies of Turkiewicz *et al.*, (2019), who clear demonstrated an antioxidant effect (ORAC) of carotenoids and terpenoids in artichoke.

The positive interaction between CQA and flavonoids on the one hand and ORAC levels on the other hand could be demonstrated in the own correlation analyses (except for 1 WAA in the first growth cycle). The results confirm that the antioxidant capacity of artichoke leaf extracts is influenced by the content of caffeoylquinic acids and flavonoids (Falleh *et*

al., 2008, Pandino *et al.,* 2015). In addition, other antioxidant compounds (carotenoids, terpenoids) are also involved in the antioxidant efficacy (Turkiewicz *et al.,* 2019).

The study conducted has shown that weedicides can cause a reduction in phenolic constituents a few days (1 WAA) after application. However, this negative effect is quickly compensated during further plant development, so that leaf yields with good qualities (measured by CQA and flavonoids) and high antioxidant activity (measured as ORAC) can be achieved in both the first and second growth cycle of the artichoke plants under moderate climate conditions.

***Conclusion*:** The current study concludes that the use of weedicide in the cultivation of leaf artichoke as a medicinal plant is possible and reasonable to achieve a good quality of the leaf extracts. Weedicide application can lead to a reduction in phenolic constituents of artichoke leaf samples; however, this negative impact is quicky compensated during further growth and development of plant under moderate climate conditions. Further field trials are required to study the degradation and metabolization of herbicidal active substances by the cultivated plants like artichoke.

Declarations:

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