Leaf Yield and Polyphenols of Artichoke (*Cynara cardunculus* L.) Influenced by Harvest Frequency and Herbicide Stress

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A thesis submitted for the requirement of doctoral degree in agriculture
from Faculty of Agricultural and Nutritional Sciences,
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Dedicated to

MY LATE UNCLE

(MAQBOOL AHMAD)

who would have been the happiest person to see me reach this level.

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LIST OF ABBREVIATIONS

% DM	Percent Dry Matter
Chl. Acid	Chlorogenic Acids
cm	centimeter
CQA	Caffeoylquinic Acids
DAA	Days After Application
DM	Dry Matter
DMC	Dry Matter per Cut
DMT	Dry Matter per Treatment
ETR	Electron Transport Rate
FAO	Food and Agriculture Organization (of the United Nations)
FM	Fresh Matter
g	gram
GLP	Green Leaves per Plant
ha	Hectares
IBS	Irritable Bowl Syndrome
LB	Leaf Blades
LSD	Least Significant Difference
LV	Leaf Veins
LYC	Leaf Yield per Cut
LYT	Leaf Yield per Treatment
NS	Non Significant
p	Probability
PAM	Pulse Amplitude Modulation
pl. m^{-2}	Plants per square meter
SC	Soluble Concentrate
SS	Stock Solution
Syn.	Synonym
t	Tones
t ha^{-1}	Tones per hectare
WAA	Weeks After Application
WP	Wetable Powder
YLP	Yellow Leaves per Plant

1. Introduction

1.1 Use and cultivation of artichoke

Artichoke (*Cynara cardunculus* L.) is a cross-pollinated and highly heterozygous plant belonging to family Asteraceae. It is an herbaceous perennial plant of Mediterranean origin, North Africa, Canary isles and Southern Europe. It is well adapted to xerothermic conditions of Southern Europe (Moglia et al., 2008; Raccuia et al., 2004), typical conditions of arid and semi arid areas of the Mediterranean environment (Bianco, 2005; Gonzales et al., 2005; Raccuia et al., 2004 and Gominho et al., 2001). Morphologically artichoke can be divided into two sub groups i.e. cardoon (syn. vegetable artichoke or cardy) featured by small floral buds and globe artichoke (syn. Artichoke) having big floral buds and feathery leaves (Hanelt, 2001). Artichoke production is an important component of regional economy in southern Europe (Moglia et al., 2008). Italy is the world's biggest producer of artichoke and produces about 470 metric tons artichoke per year (table 1). In Germany artichoke is used for the production of medicines and dietary supplements since the 70's (Schilcher, 1971). It is grown as a marginal crop in the states of Hesse, Thuringia and Bavaria where total area under the artichoke cultivation is around 100 hectares.

Table 1: Area (hectares) and Production (tonnes) of the World's top artichoke producing countries (FAO Stat 2010)

Year/ Country		Italy	Spain	France	USA	Greece	Turkey	World
1990	**Area (ha)**	48172	31100	15068	4015	3202	874	**117830**
	Production (t)	*487000*	*427900*	*97118*	*50547*	*33594*	*10000*	***1333471***
1995	**Area (ha)**	51723	18400	13899	3602	2200	1500	**112495**
	Production (t)	*517200*	*250700*	*63190*	*37149*	*24900*	*180000*	***1155577***
2000	**Area (ha)**	50283	19656	11823	3560	2800	2300	**123171**
	Production (t)	*512946*	*290189*	*63605*	*45900*	*31000*	*24500*	***1331229***
2005	**Area (ha)**	50127	18792	10179	3200	2595	2600	**125727**
	Production (t)	*469975*	*200135*	*50149*	*39420*	*27497*	*36000*	***1272505***
2009	**Area (ha)**	50700	16500	10000	3480	1800	2900	**133432**
	Production (t)	*486600*	*198900*	*45000*	*50710*	*21300*	*34859*	***1494262***

On one hand artichoke is a good vegetable known for its pleasant bitter taste and on the other hand it is an interesting and widespread herbal drug (Anonymous, 2007). Its leaf extract consists around 2 - 4 % phenolic acids (chlorogenic acid, cynarin, and caffeic acid), up to 4 % sesquiterpene lactones, and around 1 % flavonoids (scolymoside, cynaroside, and luteolin). It also contains phytosterols, tannins, sugars, starch, and inulin. Artichoke leaf extract has been used for a variety of purposes, including chronic albuminuria, hyperlipidimia, irritable bowl syndrome (IBS), jaundice and liver dysfunction. Kraft (1997) reported the use of artichoke 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 leaf extract is thought to act as a choleretic, stimulating bile flow from the liver to the gallbladder. This action is attributed to chlorogenic acid, cynarin, and scolymoside and may contribute to the extract's potential to alleviate dyspepsia. Cynaroside and luteolin may play a role in reducing cholesterol by indirectly inhibiting hydroxymethylglutaryl– coenzyme A reductase. Phytosterols may inhibit exogenous cholesterol absorption and therefore may reduce cholesterol levels.

Medicinal value of artichoke leaves have been neglected in past and a very few work has been done to device agronomic techniques to maximize the polyphenolic contents of artichoke leaves. The leaf yield is affected by different agronomic and environmental factors. Being a perennial plant artichoke grows vegetatively after the harvest and can be harvested many times during a growth period. The physiological age of the leaves makes a very big influence on the contents of polyphenols that are important for the medicinal value of artichoke. It is supposed that physiologically young leaves contain more amounts of polyphenols when compared with that of physiologically old leaves. For that reason it is very important to optimize the physiological age of the artichoke leaves for the maximum polyphenolic contents. Plant density has been recognized as a major factor for the determination of the degree of competition between plants (Tetio-Kagho and Gardener, 1988). Dry matter accumulation per unit area in maize was increased by an increase in plant population (Ma et al., 2007). Plant population per unit area too is an important factor for the optimization of polyphenolic contents of artichoke leaves. A very dense population will lead to less number of leaves per plant on the one hand and on the other hand plants will be weaker and grow taller in height (Karlen & Kasperbauer, 1987). A thin population will lead to vigorous plants with more number of leaves but there will be wide empty area that may lead to spread of weeds on one hand and on the other nutrients and moisture will be lost. Keeping the importance of plant-to-plant competition and prevalence of weeds determination of optimum planting density is very important.

Due to its rosette growth nature artichoke needs wide inter and intra row spaces. These inter and intra row spaces provide weeds a good chance to grow rapidly and compete with the crop for nutrition and space. Weeds can be controlled mechanically, chemically or biologically. Mechanical weed control is laborious and expansive where as biological control of weeds is not so common and popular among the growers. Moreover biological weed control has certain limitations as the biological weed control agents can also damage the non-target plants (Louda et al., 1997; Pemberton, 1995) and need a lot care itself in it. The best way to control the weeds is the use of herbicides that are selective (non toxic) for artichoke. Different herbicides are recommended and used for the control of weeds in artichoke. Both pre and post emergence herbicides are available for control of weeds in artichoke. Post emergence herbicides may impose a stress to artichoke either by making a layer on the leaves that may inhibit photosynthesis, affect the opening and closing of stomata, positioning of leaves and fungal attack or may affect one of the metabolic or physiological functions. This stress may be temporary or permanent as the artichoke can cover this stress 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 (Miyazawa, 2006). It is an established fact that herbicides may affect plant's physiological state by inhibiting photosynthesis or associated biochemical processes (Krause and Weis, 1984). 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 (van Coillie et al., 1983; Wong et al., 1986). Herbicides may enter plants through soil, leaves or both, but in each case they are designed to kill 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. Low photosynthetic yield will lead to minimum photosyntahtes and crop growth will be affected as crop will not get the required food that will result in low leaf yield.

Keeping the importance of herbicides and agronomic aspects like harvest frequency and plant density and their impact on artichoke the research project was designed. The project was focused on the impact of above mentioned factors on the medicinal value of artichoke. The research study was conducted to investigate the effect of herbicides, plant density and harvest frequency on leaf yield and polyphenol contents of artichoke leaves at two different locations in Germany.

1.2 Literature Review

1.2.1 Use of Artichoke

Cynara cardunculus L. (Asteraceae), commonly known as 'cardoon', is a Mediterranean species that grows naturally in harsh habitat conditions (arid region) with high temperature, elevated salinity and drought in summer (Gominho et al. 2001; Gonzalez et al. 2005). The plant is world widely used and represents an important and notable ingredient of the Mediterranean food (Fratianni et al., 2007). This multipurpose plant is used in a variety of dishes, soups and/ or salads (Gominho et al., 2000). Flowers of artichoke are traditionally used for cheese preparation (Valentao et al. 2002), whereas leaves are known for their therapeutic potential as diuretic, choleretic, and anti-diabetic and anti microbial agent particularly in folklore (Fratianni et al., 2007; Krizkova et al., 2004). The data related to antioxidant and antibacterial activities as well as phenolic composition of artichoke are scarce (Valentao et al., 2002). Artichoke can be propagated both sexually (through seed) and asexually (vegetative). Direct sowing through seed allows for regular crop rotation as crop can be used as an annual, decrease planting costs, can be sown mechanically and it eliminates the removal of off shoots.

Artichoke is a very competitive weed in itself and does not allow the mutual growth of other weeds, whereas pest and diseases do not affect its growth, that is why it can be successfully cultivated without use of agro-chemicals. Moreover, its deep and effective rooting system takes perfect advantage of the soil's inherent fertility so the crop needs nitrogen dressing only in poor soils. When it is grown during the rainy period, artichoke uses the winter and spring rains for its advantage and performs dry matter yield of around 12-16 t ha^{-1} without any irrigation. By artificial irrigation in April and May the extra yields (up to 25 t ha^{-1}) can be obtained (Grammelis et al., 2008).

Per year average production of artichoke is 20 tha^{-1}, but a dry matter of around 30-35 tha^{-1} can be obtained, consisting of about 40% stalks, 25% leaves and 35 % capitula (Fernandez, 1992 a, b; Dalianis et al., 1996). This harvested biomass is practically dry, and thus has an advantage of eliminating the drawbacks of agricultural biomass production for industrial uses, where transport, storage and conservation are affected by high moisture contents. The stalks of cardoon are about 1.5 to 1.9 m tall and branch at upper half. The stalks are circular with slightly grooved surface, and are homogeneous longitudinally without nodes. The average proportion of the pith to total cross section is 45% in area and 10% in weight. Chemically the stalks have 14.6% extractives, corresponding mainly to polar extractives, which are removed with ethanol and water (89% of the total). Lignin amounts 17% and polysaccharides 53%,

corresponding mainly to cellulose and xylan. The ash content consists of 7.7% of the dry weight of whole stalk. *Cynara cardunculus* L. has shown its potential as a fiber source for paper production. Well-delignified Kraft pulp (a technology for conversion of wood into wood pulp consisting of almost pure cellulose fibers. The process entails treatment of wood chips with a mixture of sodium hydroxide and sodium sulfide, known as white liquor that breaks the bonds that link lignin to the cellulose) with high yields can be produced using artichoke. These pulps have very low rejects, good strength properties, particularly in relation to tensile strength and moreover the energy requirements for refining are very low. Pulp produced from depithed stalks is of better quality than that of stalks with presence of pith parenchyma (Gominho et al., 2001)

Walker et al. (2001) reported that artichoke (*Cynara scolymus* L.) leaf extract has been shown to reduce symptom severity in a subset of patients with dyspepsia identified as suffering from irritable bowl syndrome (IBS). Bundy et al. (2004) conducted a study with otherwise healthy adults (208 persons) suffering dyspepsia. The volunteers were assigned randomly 320 or 640 g (1or 2 capsules) of a standardized (1:5) aqueous full spectrum extract of artichoke leaves for two months to be taken on daily basis. At the end the volunteers were accessed for IBS incidence through a questionnaire to be filled by them. The study reported that IBS incidence decreased significantly by 26.4 % after the treatment with artichoke leaf extract for two months, with 55 volunteers falling outside the Rome criteria for IBS ($p < 0.001$). More than half of the subset reported a usual pattern “alternating constipation/diarrhea” after the medication period. However, after the treatment, there was a significant shift in usual bowl pattern toward the ‘normal’ ($p < 0.001$) no significant differences were found between the dose groups.

Artichoke leaf extract and its components are also used as antimicrobial agents against certain bacterial strains (Mossi and Echeverrigaray, 1999). Zhu et al. (2004) studied the antimicrobial activities of artichoke leaf extract. Gram-positive bacteria, gram-negative bacteria, yeasts and molds were used for antimicrobial activities studies. Three fractions of artichoke leaf extract were used for preliminary disk assay. The results showed that *n*-butanol fraction, followed by chloroform and ethyl acetate fractions, exhibited most significant antimicrobial activities. The study showed that at least four gram-positive bacteria and two gram-negative bacteria were found susceptible to artichoke leaf extracts. Five fungi including three yeasts also showed susceptibility to all three fractions of artichoke leaf extract.

1.2.2 Phenolic compounds

Leaf yield and polyphenols in artichoke (*Cynara cardunculus* L.) are affected by different cultivars and this affect is different during different growing seasons depending upon the environmental (air temperature and precipitation) conditions (Honermeier et al., 2009). Cultivars of ‘cardoon group’ produced maximum leaf area index (LAI) and leaf yield during all the years in a study conducted for the period of 2001 to 2003 in Germany. The research revealed that during first two experimental years (2001 and 2002) caffeoylquinic acids (CQA) concentration of leaves were higher, whereas during the third year (2003) were lower. In contrast to 2001 and 2002, higher concentration of polyphenols was detected during the second harvest in comparison with that of first harvest in 2003. A positive correlation between the caffeoylquinic acids and flavonoids was observed in the study (Honermeier et al., 2009).

In a study focusing the stress-induced biosynthesis of di caffeoylquinic acids in globe artichoke 8, 10, 12 and 14 weeks old leaves were collected from 'Orlando' and 'Violetto di Sicilia' varieties of artichoke grown in environmentally controlled climatic chambers. 2 cm floral disks were removed from 10-12 weeks old leaves and were stored in liquid nitrogen at –80 °C until further analysis. Detached floral disks were exposed to UV-C (254 nm) light for 20 min, through a 16 W germicidal lamp at a distance of 20cm. Controls were treated in another chamber with the same conditions but with lamp off. The finely ground samples were extracted by methanol through sonication and then separated by centrifugation and at the end analyzed with HPLC using reversed phase chromatography with photodiode array (PDA). The first peak (retention time 14.5 min) was identified as chlorogenic acid (5- caffeoylquinic acid) having maximum wavelength absorbance (λ_{max} 324 nm). Peaks 2 (15.0 min) and 3 (17.3 min) also presented caffeoylquinic acids, having similar λ_{max} (324 nm) whereas peak 4 (25.8 min) was identified as luteolin-7-glucoside by comparison of retention time and λ_{max} (343 nm). Peaks 5, 6, 7 and 8 all were identified as different caffeoylquinic acids. A significant increase (4-fold when compared with control, p = 0.017) was observed in the main content of the di caffeoylquinic acid isomer II upon UV-C treatment. The same treatment also slightly influenced the levels of chlorogenic acid, caffeoylquinic acid isomer II, the minor di caffeoylquinic acid isomer I, and luteolin-7-glucoside, but the observed increases were not statistically significant (chlorogenic acid p = 0.33; luteolin-7-glucoside p = 0.457). In another experiment, the specific effect of UV-C irradiation on di caffeoylquinic acids synthesis was confirmed in other genotypes of 'Orlando' and 'Violetto di Sicilia'. A marked induction of chlorogenic acid and main di caffeoylquinic acid isomer II was observed in all tested genotypes (Moglia et al., 2008)

Polyphenolic contents of a plant depend on a number of intrinsic (genetic) and extrinsic (environmental, handling and storage) factors (Fratianni et al., 2007; Rapisarda et al., 1999). Irrespective of different plant organs polyphenolic contents in artichoke are higher than that of several other species cited in literature. Djeridane et al. (2006) concluded that abundance of polyphenols in artichoke is characteristic of the asteraceae family. It may be related to the hard climatic conditions of the usual habitat of the members of asteraceae (hot temperature, high solar exposure, drought, salinity etc.), which stimulate biosynthesis of secondary metabolites such as polyphenols.

Phenolic compounds in the aerial parts (leaves, flowers and seeds) of cardoon differ depending on their type. In a study conducted during 2005 in Tunis to clarify the difference in these parts, highest yield was registered in the leaves (ca. 35 %), while that of seeds and flowers was 3.5 and 4.6 times lower, respectively. All these organs exhibited high polyphenol content, comprised between 7.5 and 15.0 mg GAE g^{-1} DW (milligram gallic acid equivalent per gram dry weight) where leaves and seeds constituted statistically same polyphenol contents. Flavonoid content in the studied organs ranged form 6 to 10 where maximum flavonoids content were found in leaves and were statistically similar to that of seeds but significantly higher with that of flowers. Flavonoids comprised major portion of the total polyphenol contents (80 %) in flowers whereas it was found lower in seeds and leaves (68 % and 61 %, respectively). Depending on the retention times of the calibration standards fifteen phenolic compounds including gallic, sinapic, chlorogenic, syringic, vanillic, rosmarinic, trans-cinnamic, ferulic and p-coumaric acids as well as epicatechin, quercetrin, quercetin, apigenin, amentoflavone and flavones. The analysis of the typical HPLC chromatogram depicted the syringic and trans-cinnamic acids were

major leaf phenolic acids and epicatechin and quercetrin as major flavonoids (Falleh et al., 2008).

Artichoke leaves contain a variety of 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 (Grancai et al., 1994; Slanina et al., 1993; Seabra et al., 2002). *Cynara cardunculus* L. is most commonly used as antioxidant (Valentao et al., 2002). Halliwell et al. (1995) reported that antioxidants are very important as these may help to protect the body against damage by reactive oxygen species (ROS). Valentao et al. (2002) quantified phenolic compounds in artichoke leaves and found that luteolin-7-glucoside was the compound present in higher amounts (1290 mg kg^{-1}), followed by 5-caffeoylquinic acid (380 mg kg^{-1}) and 1,5-dicaffeoylquinic acid (120 mg kg^{-1}). Cynarin and apigenin 7-glucoside exhibited lower amounts (70 and 20 mg kg^{-1}, respectively). These results suggested that luteolin-7-glucoside most probably is the major compound contributing the antioxidant properties exhibited by infusion. The authors, by the presence of phenolic compounds in lyophilized infusion of cardoon, concluded that scavenging activities observed against superoxide radical, hydroxyl radical and hypochlorous acid is perhaps due to these compounds that contribute to the protective effects observed in the study.

Fratianni et al. (2007) studied the phenolic content of the heads and leaves of five different varieties of artichoke and one cultivated accession of cardoon. The researchers concluded that heads contain higher total polyphenols than that of leaves. Romani et al. (2006) also reported that heads of globe artichoke variety 'Violetto di Toscana' have higher phenolic contents than that of leaves. These results suggest that in addition to leaves that are widely used for pharmaceutical purposes (Gebhardt, 1997) artichoke heads can also represent an important source of polyphenols (Kraft, 1997; Llorach et al., 2002).

Schutz et al. (2004) developed a method for identification and quantification of phenolic compounds in artichoke and detected 11 caffeoylquinic acids and 8 flavonoids. The researchers found that recovery rate for phenolic acids as 87 % for caffeic acid and 95 % for both 5-*O*-caffeoylquinic acid and 1, 3-di-*O*-caffeoylquinic acid. The recovery for 7-*O*-glucoside was found to be 88 %. The recovery for narirutin and apigenin 7-*O*-glucoside was 91%. Due to the availability of limited standard/reference substances the authors used LC-MS (HPLC coupled with mass spectrometry -Liquid chromatography Mass spectrometry) for the assignment of peaks and further characterization of individual substances. The individual substances identified through the peaks included: 1-*O*-caffeoylquinic acid (pseudochlorogenic acid), 3-*O*-caffeoylquinic acid (neochlorogenic acid), 4-*O*-caffeoylquinic acid (cryptochlorogenic acid), luteolin 7-*O*-glucuronide, apigenin 7-*O*-glucuronide, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, 1,5-di-*O*-caffeoylquinic acid, luteolin 7-*O*-rutinoside, apigenin 7-*O*-rutinoside and naringenin 7-*O*-glucoside.

1.2.3 Chlorophyll fluorescence

Chlorophyll fluorescence based methods have been used to study the effects of environmental factors and herbicides on plants. Chlorophyll fluorometers were originally designed to investigate photosynthetic processes associated with primary photochemistry and electron transport. Use of chlorophyll fluorescence methods for

assessment of the inhibitory effect of herbicides affecting photosynthetic electron transport in ecotoxicological research, therefore, is not surprising.

Photosynthetic attributes vary among the leaves of different species, age of leaves and the environmental light. Within the crown, the plants in order to attain maximum carbon gain by the use of limited resources, particularly nitrogen, arrange the leaves with high photosynthetic activity under sunny conditions and the leaves with low photosynthetic activity under shade (Field, 1983; Hirose and Werger, 1987). Investigation of photosynthetic rates and photosynthetic capacity can be carried out through two different methods. In the first method gas exchange system can be used to measure the light-saturated rate of the net photosynthetic carbon assimilation per unit leaf area. Net photosynthetic carbon assimilation rate has been used as a parameter for photosynthetic rate, which on one hand is known to be correlated with relative growth rate (Lusk and Del Pozo, 2002) and on the other hand depends on successional stage of species (Koike, 1990). The second method uses chlorophyll fluorescence measurement system (Genty et al., 1989) that measures electron transport rate (ETR) at light saturation that is also known as photosynthetic capacity. Most of the researchers use chlorophyll fluorescence measuring system and measure electron transport rate (ETR) to express photosynthetic rate physiological attributes of focal leaves.

Miyazawa and Yahata (2006), in order to examine the reliability of carbon assimilation rate method on the ETR values and carbon assimilation rate-ETR relationship, compared photosynthetic carbon assimilation rate and simultaneously measured electron transport rate (ETR) through photosystem II, under field conditions. The authors concluded that C_i {pCO_2 of the intracellular space of the leaf (Pa)} changed during the daytime at saturated light and was different among different leaves with an average C_i value of 21 Pa. With respect to biochemical simulations, maximum rate of RuBP carboxylation and ETR increased by increasing leaf temperature until peak values were attained. As the specific factor of Rubisco to CO_2 decreased with a decrease in leaf temperature, light saturated rate of net photosynthesis (net carbon assimilation rate, µmol m^{-2} s^{-1}) reached its maximum level at lower leaf temperatures and decreased with higher leaf temperatures. Electron transport rate (µmol m^{-2} s^{-1}) that was calculated by biochemical methods showed the similar dependence to leaf temperature. The values of net carbon assimilation rate were positively correlated with ETR values, but due to large variations in the ETR values it was not possible to estimate net carbon assimilation rate by using ETR values. No clear differences were obtained in net carbon assimilation rate- ETR during the months of August and November, whereas in January the data at lower leaf temperature showed a steeper slope (high net carbon assimilation rate/ETR values) than the data collected at higher leaf temperatures (lower net carbon assimilation rate/ ETR values). The authors, although observed a positive correlation between ETR and net carbon assimilation rate in model simulation based on several assumptions but concluded that estimation of net carbon assimilation rate based on ETR values is difficult under field conditions.

The previous studies have shown that measurement of chlorophyll *a* (Chl *a*) fluorescence in plants is an efficient tool for studying photosynthesis (Papageorgiou, 1975; Lavorel and Etienne, 1977). It has been established, after thorough studies, that dissipated chlorophyll fluorescence associated to photosynthesis can be used as a simple and rapid method to study plant physiological state (Krause and Weis, 1984; Lichtenthaler and Rinderle, 1988; Bolhar-Nordenkampf et al., 1989). As many herbicides affect photosynthetic processes, chlorophyll fluorescence offers provides

higher sensitivity compared to that of, for instance, biotests based on inhibition of growth (El Jay et al., 1997). Some previous studies have reported the advantages of using chlorophyll fluorescence as a biotest (Lichtenthaler and Rinderle, 1988; Rohacek and Bartak, 1999; Tomlin, 2000).

For the last tow 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 (table 2).

Table 2: Some useful fluorescence parameters obtained by PAM-fluorometry.

Parameter	Equation	Reference
Φ_M	$(F_M - F_0) / F_M$	Kitajima and Butler, 1975
Φ'_M	$(F'_M - F_S) / F'_M$	Genty et al., 1989
qP	$(F'_M - F_S) / (F_M - F'_0) (F'_0 - F_S)$	Schreiber et al., 1986
qL	$(F'_M - F_S) / (F_M - F'_0)$	Kramer et al., 2004
qN	$1- [(F'_M - F'_0) / (F_M - F_0)]$	Schreiber et al., 1986
NPQ	$(F_M - F'_M) / F'_M$	Bilger and Bjoerkman, 1990
$q_{p\ (REL)}$	$(F'_M - F_S) / (F_M - F'_0)$	Buschmann, 1995
$Q_{N\ (REL)}$	$(F_M - F'_M) / (F_M - F'_0)$	Buschmann, 1995
$UQF_{\ (REL)}$	$(F_S - F'_0) / (F_M - F'_0)$	Juneau et al., 2005

Inhibition of metabolic reactions that are not directly involved in photosynthesis modifies the pool size of many metabolic intermediates, which could produce a feed back effect on photosynthesis and as a result affect fluorescence emission (Owens, 1991). So chlorophyll fluorescence can also be efficiently used to study the effects of herbicides affecting metabolic processes that are not directly linked to photosynthetic electron transport.

1.2.4 Herbicides

Over the last half of the century, increasing land superficies devoted to agriculture has resulted in an exponential escalation of herbicide application. It was thought to be necessary in order to reduce labor and energy requirements in crop production (Lockhart et al., 1990). As a consequence a huge amount of toxic compounds enter into soil and ultimately reaches streams and underground water reservoirs and eventually reaches to the nearby lakes and rivers (Frank and Logan, 1988).

Independent of the entering path (soil, foliage or both), herbicides are designed to kill unwanted plants either by inhibition of photosynthesis or by affecting any of the metabolic processes (Tomlin, 2000).

Action of most of the foliar applied herbicides is closely related to their absorption, for both contact and systemic herbicides that results in translocation to other parts of plant, where they play their role on specific sites. Many factors related to leaves, environment, herbicide formulation and application solution may affect this absorption process (Durigan-Marcel, 2005).

Paynter et al. (2004) conducted a nationwide survey to study the weed biocontrol and its impact on non-target plants. The survey focused on studying the non-target damage caused by 20 biological agents used to control weeds in New Zealand. The survey reported that most of the studied biological agents (16) proved apparently host-specific, whereas two species (*Tyria jacobaeae* and *Phytomyza vitalbae*) were found to be attacking the native plants, although the attack was minor and predictable from host-range testing performed prior to release. For the other remaining two species (*Bruchidius villosus* and *Cydia succedana*) non-target attack was not predictable from host-range testing. The larvae of these species mainly confined closely to their target plants.

Hendrickson et al. (2004) concluded that combined measurements of chlorophyll fluorescence and gas exchange are useful parameters for distinguishing stomatal versus non-stomatal effects on one hand and on the other hand for estimation of importance of various types of energy use like thermal dissipation and photorespiration.

Chlorophyll fluorescence analysis is a sensitive and early indicator of damage to photosynthetic apparatus (Krause and Weis, 1991; Schreiber et al., 1994). Toxic effects of the herbicide diuron on plant photosynthesis are well established (van Rensen, 1989). Herbicide inhibits the photoreduction side of photosystem II (PSII) by binding with high affinity at Q_B-binding site of PSII photosynthetic complex and preventing Q_B from binding at this location. Haynes et al. (2000) studied the effect of herbicide diuron on three species of sea grass. The researchers used diving PAM fluorometer for assessing the photosynthesis by measuring change in chlorophyll fluorescence. The study reported that all three species of sea grass used for the study exhibited a rapid fluorescence response to the applied herbicide. When these species were returned to the clean water the recovery process was initially rapid in all three species, but this recovery process was not necessarily sustained while all species showed fluctuations in a 5-day recovery period.

1.2.5 Harvest frequency

Growth period (harvest frequency) significantly affects the yield of globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori]. The head weight of the F_1 hybrid of artichoke decreased in general when harvest time was delayed from November to April. The pattern was found to be linear and had greater magnitude in ‘Orlando’ than that of ‘Violetto di Sicilia’ (vegtatively propagated variety mainly grown in Italy). The head length and width values varied little when harvested was delayed from November to February but showed a clear and significant increase form February to April. Anticipation of harvest period increased the L/W ratio of the ‘Orlando’ heads linearly (Mauromicale and Ierna, 2000).

Mauromicale and Ierna (1995) studied the combination between early sowings and treatments of gibberellic acid on seed grown globe artichoke (*Cynara scolymus* L.). The authors reported that F_1 hybrid ‘Orlando’ substantially modified the harvest period by combination of early sowing and appropriate doses of gibberellic acid. The effectiveness of gibberellic acid proved greater in a combination with earlier sowing date.

Matthes and Honermeier (2007) studied the effect of harvest date and nitrogen fertilizer on leaf yield and polyphenolic contents of artichoke leaves (varieties Green Globe and White Giant). The research study was conducted at the experimental

research station Giessen, Germany for a time period of 2003 – 2004. For both the years the crop was divided into three growth phases and during every growth phase it was harvested more than once. During the first year the research study the crop was harvested weekly (but results were given only fortnightly) and for the second year of the experiment the crop was harvested every two weeks resulting in 15, 11 and 5 harvests for first, second and third growth phases (Green Globe), respectively for 2003 and 5, 4 and 1 for 'Green Globe' and 6, 4 and 1 harvests for 'White Giant', respectively in 2004. The results of the experiments show that during first growth phase in 2003 concentration of chlorogenic acids was found maximum in case of first harvest (48 days of vegetation) and then went on decreasing towards the latter harvests with the minimum concentration of chlorogenic acids to be observed at 15th harvest (146 days of vegetation) of the artichoke crop. Concentration of cynarosides showed a different trend to the harvest dates, where maximum concentration of cynarosides was observed in case of third harvest (62 days of vegetation) and it went on decreasing afterwards till 13th harvest (118 days of vegetation) and then started increasing till 15th harvest (146 days of vegetation). Dry matter yield in this case increased with increasing the vegetative growth period and was found maximum at 5th harvest where crop completed 76 days of vegetative growth and sustained till 92 days of vegetation. Minimum dry matter yield during the first growth phase was observed in case of the longest growth period (146 days of vegetation). During the second growth phase a different trend was shown by the artichoke leaves, where dry matter yield of artichoke leaves decreased with increasing the vegetative growth period and in contrast to it the poly phenolic contents increased. Both dry matter yield and polyphenolic contents of the artichoke leaves were not affected significantly by different harvest dates used during the course of the study. Dry matter yield for both the varieties studied during first growth phase of 2004 increased by increase in vegetative growth period of the crop, but during the second growth phase it showed a different trend, where for 'Green Globe' it increased till 3rd harvest and then decreased onwards, whereas in case of 'White Giant' it increased till 2nd harvest and then decrease with increasing vegetative growth period. The authors reported that flavonoids were not affected by different harvest dates in both the varieties during 2004. Caffeoylquinic acids during first growth phase of 2004 increased with the increase in vegetative growth period of 'Green Globe' and during the second growth phase it increased till 2nd harvest (highest concentration of CQA) and then decreased till 4th harvest and showed a higher CQA content at 5th harvest. CQA contents of 'White Giant' during first growth phase showed a mixed response to the harvest dates, which were found lowest at 2nd harvest and for all other harvests showed a statistically at par response. During the second growth phase it increased from 2nd harvest towards 4th harvest (highest CQA).

1.2.6 Plant density

Plant density is perhaps the most important factor that determines the degree of competition among plants (Tetio-Kagho and Gardner, 1988). Elia et al. (1991) studied the effect of sowing date, plan density and nitrogen rate on artichoke (*Cynara scolymus* L.). The researchers used five different planting densities in sub plots against three sowing dates in main plots. The study found that increasing number of plants per unit area decreased number of heads per plant, while it increased yield per unit area.

Plant density can affect different parameters like, architecture of the plant, size, germination percentage and yield of the seeds in many species like carrot (Gray et al., 1983), and fennel (Damato et al., 1994). Recent research studies have shown that an in increase in plant density in fennel, carrot and chicory decreases number of flowers and yield per plant and increases number of flowers per unit area. An increase in plant density improves the seed quality in fennel and carrot.

Damato et al. (2006) conducted a research to study the effect of plant density and off shoot removal on the seed yield and quality of artichoke. Three plant densities (0.8, 1.0 and 1.3 plants m^{-2}) were used in the research study conducted during 2000-2002. Authors reported that plant density had a slight influence on both yield components and yield of artichoke, where increases in plant density significantly decrease the number of achenes per lateral head. In this case plant density did not affect the seed yield per central and lateral head significantly during 2001. Both the studied factors had a non significant effect on the yield and yield components of artichoke during the year 2002. The study also reported that achene yield per hectare of the central head decreased with an increase in plant density (71 vs. 42 kg ha^{-1} at lowest and highest plant density, respectively).

Architecture of flowering plants is determined by the degree of branching, internodal elongation and shoot dormancy (Ward and Leyser, 2004). Raccuia and Melilli (2006) studied the effect of plant density on plant architecture and biomass partitioning of artichoke (*Cynara cardunculus* L. var. *sylvestris* Lam.) in Italy during 2002-2003. Four different plant densities i.e.1, 2, 4 and 8 plants m^{-2} were used, where seed propagated wild cardoons with 3 – 4 leaves were transplanted in September 2002 with three replications. The obtained results show that plant density affected the architecture and biomass partitioning of artichoke plants. Maximum number of branches in this case was observed at the lowest plant density and these decreased towards lower plant spacing (higher plant densities) and this decrease was mainly due to less number of secondary branches. In case of partitioning of biomass, 1 and 2 plants m^{-2} showed statistical similarity with each other, where above ground biomass resulted 63 % of total biomass, while roots represented 37 % of it. On the contrary an increase in the plant density (statistically at par at 4 and 8 plants m^{-2}) reduced the above ground biomass, i.e. 57 % and 43 % aboveground and underground of the total biomass, respectively. Regression between the grain production and plant density showed that higher plant density reduced the production of grains in artichoke. High correlation coefficient (r^2= 0.995) between the grain production and branches per plant showed that grain production was maximum at lower plant density. Maximum grain production per hectare in this case was recorded by the plant density of 8 plants m^{-2} (0.84 t ha^{-1}).

1.3 Hypotheses

In several studies it could be found that non biotic stress and different plant densities can affect the leaf yields and quality of globe artichoke. Most of these studies were carried out under Mediterranean conditions with globe artichoke as a vegetable crop. These results are transferable neither to humid conditions in Germany nor to cultivation of artichoke as a leaf used crop for medicinal purposes. For that reason field experiments were carried out to clarify the effect of herbicide stress and plant density on leaf yield and content of polyphenolic compounds in the leaves of artichoke in Germany.

The following hypotheses were made for the research study:

1. Plant density and harvest frequency affect leaf yield of artichoke.
2. Polyphenols increase by higher harvest frequency of artichoke leaves.
3. Herbicides affect yield and content of polyphenolic compounds of artichoke leaves.
4. Herbicide stress is detectable by non destructive chlorophyll fluorescence measurements.
5. Analysis of polyphenolic compounds through HPLC can identify herbicide stress in artichoke leaves.

2. Materials and Methods

2.1 Overview of the experiments

Two different field experiments to study the effect of different agronomic and a-biotic factors on growth, yield and quality parameters of artichoke were carried out for three consecutive years i.e. 2006 to 2008. These experiments were carried out at research stations of the Institute of Agronomy and Plant Breeding I in Giessen and Gross Gerau. The field experiment in Gross Gerau was focused on the effect of different plant densities and harvest frequencies on the plant growth, leaf yield and polyphenol concentration of artichoke. The effect of different herbicides was studied in the experiment held at Giessen, where for the first two years (2006 & 2007) five different post emergence herbicides and during the third year (2008) eight different post emergence herbicides were used against a manually weeded control. An overview of all the experiments conducted for the study is arranged in table 3.

Table 3: An overview of the experiments conducted during 2006 to 2008

Year	Location	Study Factor	Treatments
2006	Gross Gerau	Plant Density & Harvest Frequency	4, 8, 12 & 16 plants m^{-2} LHF, MHF & HHF (3, 5 & 6 Harvests, consecutively)
2007	Gross Gerau		
2008	Gross Gerau		
2006	Giessen	Herbicides	Control, Haloxyfop, Phenmedipham, Pyridate, Quizalofop & Prosulfocarb
2007	Giessen		
2008	Giessen		Control, Carfentrazone, Phenmedipham, Pyridate, Quizalofop, Prosulfocarb, Rimsulfuron, Aclonifen & Clomazone

2.2 Harvest frequency field experiments

2.2.1 Soil conditions

The experimental site is situated approximately 2.0 km North East of the town Gross Gerau with a latitude of 49° 45´ North and a longitude of 8° 29´ East and an altitude of 90 meter above sea level. The top layer (0-25 cm) of the soil is crumb, of diluvial nature and contains sandy soil, which is weakly humic with low buffering capacity. The soil from 50 cm depth (downwards) is loamy (to clay) with red underlying layer (Sprendlinger forest) partially cemented with gravels to form a solid bracing. Secondary calcareous deposits are also found in the form of different layers of

Table 4: Soil conditions in harvest frequency field experiments with artichoke Gross Gerau 2006 – 2008

Parameter	Unit	2006	2007	2008
Nmin [1)]	kg ha^{-1}	18 (24.02.2006)	18 (19.02.2007)	17 (18.02.2008)
pH		6.3	6.6	6.4
P	mg $100g^{-1}$	20.0	26.0	34.0
K	mg $100g^{-1}$	15.0	20.0	22.0
Mg	mg $100g^{-1}$	5.0	7.0	4.0
B	mg $100g^{-1}$	0.36	-	0.16
P-/ K-fertilization				
P	kg ha^{-1}	31 (27.02.2006)	24 (10.03.2007)	31 (20.02.2008)
K	kg ha^{-1}	174 (27.02.2006)	134 (10.03.2007)	174 (20.02.2008)

[1)] Nmin: NO_3^-- N + NH_4^+-N in 0 – 90 cm soil depth

different thickness at different depths of the soil (up to 1.0 meter). The deep layers of the soil contain partially backed fine sand.

Representative soil samples were analyzed for concentration of mineral nitrogen, phosphorous, potassium, magnesium and boron as well as pH value before sowing of the artichoke. At soil preparation phosphorous and potash were applied to the soil as basal dose. Table 4 depicts the details of the soil analyses and the basal fertilizer dose.

2.2.2 Climate conditions

The climatic data (air temperature and precipitation) at the experimental location for the growth period of artichoke (April to October) for all three years is presented in figures 1 to 3.

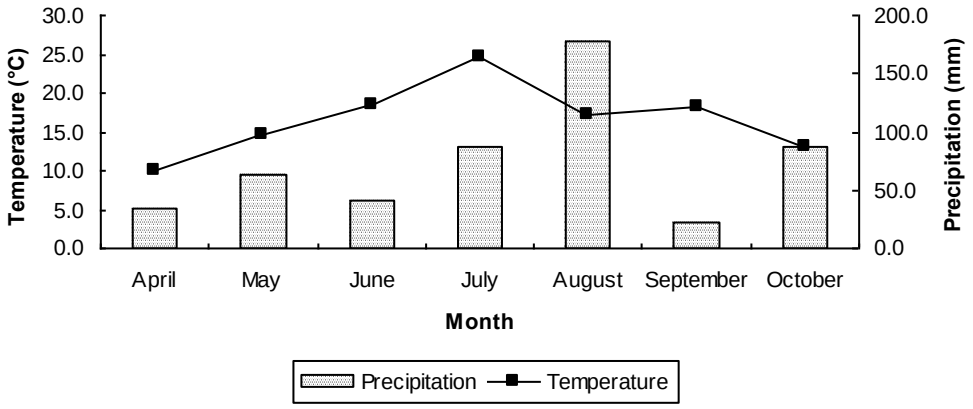


Figure 1: Air temperature (°C) and precipitation (mm) during the growth period of artichoke Gross Gerau 2006

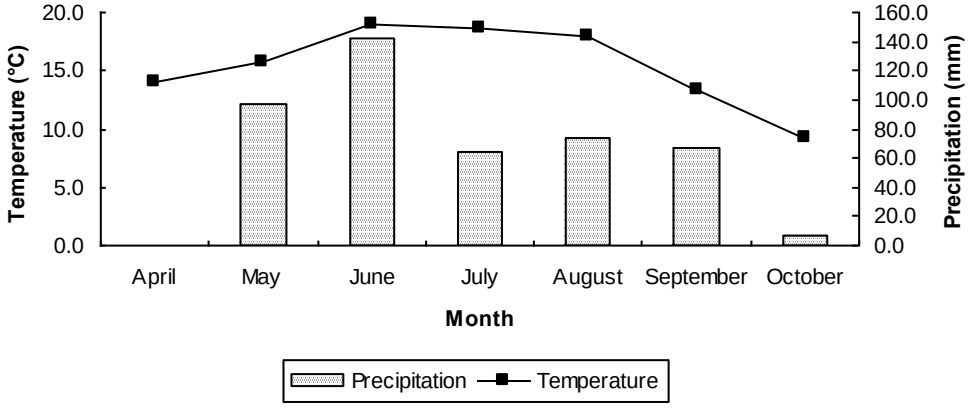


Figure 2: Air temperature (°C) and precipitation (mm) during the growth period of artichoke, Gross Gerau 2007

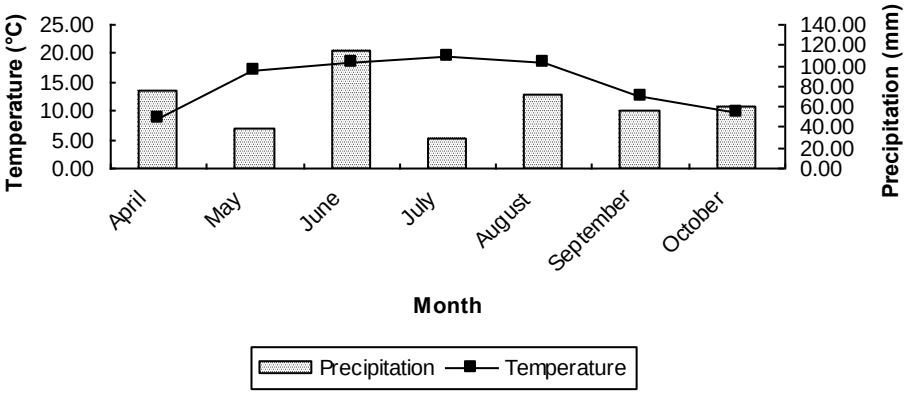


Figure 3: Air temperature (°C) and precipitation (mm) during the growth period of artichoke, Gross Gerau 2008

2.2.3 Design of the experiment

Quadruplicated experiment was carried out in randomized complete block design (RCBD) in factorial arrangement. Gross plot size was maintained as 3.0 x 7.0 m^2 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 apart rows. The cultivar Gobbo di Nizza (cardoon type from Italy) was sown manually at approximately 2 cm soil depth on 20.04.2006, 17.04.2007 and 22.04.2008 respectively for the three growing seasons.

Two factors studied included planting density and harvest frequency

Factors:

A) Planting Density:

Factor	Planting Density
A_1	4 plants m^{-2}
A_2	8 plants m^{-2}
A_3	12 plants m^{-2}
A_4	16 plants m^{-2}

B) Harvesting Frequency:

Factor	Harvesting Frequency
B_1	First harvest just before the initiation of budding, by usual harvesting method (3 harvests)
B_2	First harvest before B_1, at development of leaf rosette. Then after every 4 weeks (5
B_3	First harvest at the same time as B_2. Then after every 3 weeks (6 harvests)

Plants were harvested manually at 5 cm height from soil according to the treatments and samples were stored for further analyses. 40 kg N ha^{-1} was applied after sowing and same dose was applied after every harvest as shown in table 5.

Table 5: Nitrogen fertilization in harvest frequency field experiments with artichoke Gross Gerau 2006 – 2008

Year	2006			2007			2008		
Treatment	**HF1**	**HF2**	**HF3**	**HF1**	**HF2**	**HF3**	**HF1**	**HF2**	**HF3**
	kg N ha^{-1}								
	40	40	40	40	40	40	40	40	40
	-	40	40	-	40	40	-	40	40
	40	-	40	40	-	40	40	-	40
	-	40	-	-	40	-	-	40	-
	-	-	40	-	-	40	-	-	40
	-	40	-	-	40	-	-	40	-
	40	-	40	40	-	40	40	-	40
	-	40	40	-	40	40	-	40	40
N total	**120**	**200**	**240**	**120**	**200**	**240**	**120**	**200**	**240**

A bird's eye view of the harvest frequency experiment for all three years of experimentation is presented in table 6, which states the sowing, germination and harvest dates and other relevant details regarding the growth period (days) of each harvest and whole growth period including all harvests within a harvest frequency.

Table 6: Plant development and harvest times in harvest frequency field experiments with artichoke, Gross-Gerau 2006 – 2008

	2006			2007			2008		
	HF1	**HF2**	**HF3**	**HF1**	**HF2**	**HF3**	**HF1**	**HF2**	**HF3**
Sowing Date	20. 04. 2006			17. 04. 2007			22. 04. 2008		
Germination (Date)	05. 05. 2006			02. 05. 2007			02. 05. 2008		
	Number of harvests/ growth period of the plants								
	-	1/60	1/60	-	1/61	1/61	-	1/62	1/62
	1/81*)	-	2/22	1/83	-	2/22	1/81	-	2/19
	-	2/29	-	-	2/29	-	-	2/27	-
	-	-	3/20	-	-	3/21	-	-	3/22
	-	3/27	-	-	3/29	-	-	3/28	-
	2/42**)	-	4/21	2/43	-	4/22	2/42	-	4/20
	-	4/28	5/21	-	4/28	5/21	-	4/28	5/22
	-	-	6/21	-	-	6/20	-	-	-
	3/50	5/29	-	3/54	5/33	-	3/56	5/34	6/34
Harvests (No.)	3	5	6	3	5	6	3	5	6
Growth Period (days)	173	173	165	180	180	167	179	179	179

HF: Harvest Frequency.
*): 1/81= 1st harvest/ 81 days after germination.
**): 2/42 = 2nd harvest/ 42 days after the previous harvest

Plant density was maintained by manual sowing of the seeds and two seeds per hole were sown to avoid the missing plants. The double germinated plants were eradicated after the germination of the seeds. Plant population per unit area was recorded before the harvest of the artichoke crop. Table 7 describes the success of maintenance of the plant population per unit area for all three years.

Table 7: Plant population per unit area (m^2) obtained for different plant densities of artichoke in Gross-Gerau, 2006 – 2008

Plant density (Pl. m^{-2})	Plant populations (m^2)		
	2006	2007	2008
4	3.95	3.95	3.96
8	7.90	7.86	7.90
12	11.75	11.77	11.84
16	15.52	15.39	15.70

2.2.4 Study Parameters

Plant height (cm) was measured before the harvest of the crop. Height was recorded from the ground level to the tip of the longest leaf with the help of meter rod. The plant height was recorded for 10 plants from the two middle rows (1, 3, 5, 7 and 9 plants from one row and 2, 4, 6, 8 and 10 plants from the second row) of the plots and the average was worked out. Two middle rows of the plots were counted and plant population in plants m^{-2} was calculated. The data regarding plant cover (%) were collected by visual observation depending on the ground area covered by the artichoke leaves. The data were expressed in percent. Green and yellow leaves per plant were calculated by counting total number of green and yellow leaves from all plants in the two middle rows and average was worked out. Fresh leaf yield (t ha^{-1}) was calculated by harvesting all plants from the two middle rows of all plots and the weight was recorded in Kg. The recorded weight was then converted to t ha^{-1} for final manipulation of data. Two samples of fresh leaves were taken for Dry matter estimation from each plot. The leaf samples were oven dried at 105 °C for a constant dry weight. Percent dry matter was recorded by using the following formula and then average was worked out.

$$\text{Dry Matter (\%)} = \frac{\text{Weight of dried sample (g)}}{\text{Weight of fresh sample (g)}} \times 100$$

Dry matter (t ha^{-1}) was calculated as under;

Dry Matter (t ha^{-1}) = Fresh leaf yield (t ha^{-1}) x Dry matter (%)

2.3 Herbicide field experiments

2.3.1 Soil conditions

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. Humus contents of the soil range from 1.5 % to 4.0 %. The nutrient content and pH value of the soil is to find in table 8. 80 kg N ha^{-1} was applied as basal dose at the time of seedbed preparation.

Table 8: Soil conditions in herbicide field experiments with artichoke, Giessen 2006 – 2008

Parameter	Unit	2006	2007	2008
Nmin [1)]	Kg ha^{-1}	36.82 (13.03.2006)	35.05 (15.03.2007)	34.04 (14.02.2008)
pH		6.6	6.3	6.5
P	mg $100g^{-1}$	26.48	26.1	14.9
K	mg 100g	15.04	11.6	6.41
Mg	mg 100g	-	12.3	27.4
B	mg 100g			

[1)] Nmin: NO_3^-- N + NH_4^+-N in 0 – 90 cm soil depth

2.3.2 Climate conditions

The climatic data at the experimental station Giessen for the growth period of artichoke (April to October) for all three years are presented in figures 4 – 6.

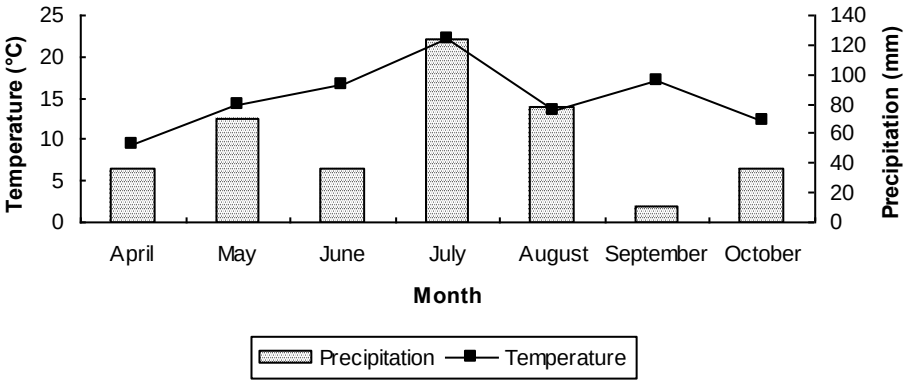


Figure 4: Air temperature (°C) and precipitation (mm) data during the growth period of artichoke, Giessen 2006.

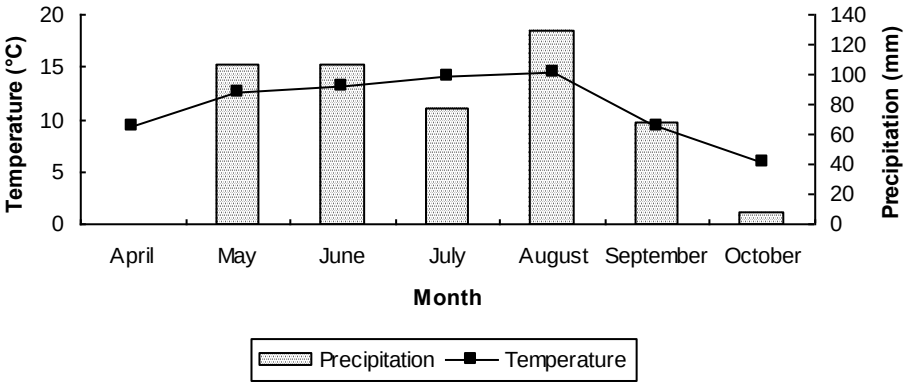


Figure 5: Air temperature (°C) and precipitation (mm) data during the growth period of artichoke, Giessen 2007.

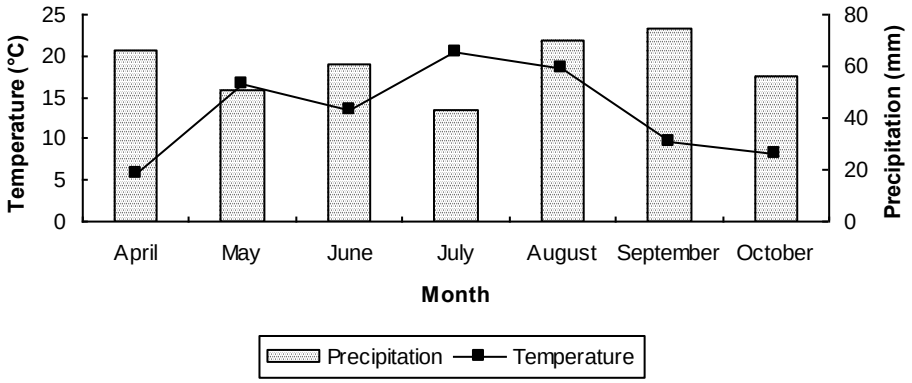


Figure 6: Air temperature (°C) and precipitation (mm) data during the growth period of artichoke, Giessen 2008.

2.3.3 Design of the experiment

The herbicide experiment was designed in randomized complete block design (RCBD) with four replications. Gross plot size was maintained as 3.0 x 7.0 m^2 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 apart rows. Gobbo di Nizza cultivar of artichoke (cardoon) was sown manually at approximately 2 cm soil depth on 08.05.2006, 16.04.2007 and 28.04.2008 respectively, for all three years. Five different post emergence herbicides were used against a manually weeded control for the years 2006 and 2007, whereas during 2008 eight herbicides were used (table 9).

Table 9: Herbicidal treatments used in the field experiments with artichoke, in Giessen 2006 – 2008

Treatment	Herbicides		
	Trade Name	Active Ingredient	Dose (L ha^{-1})
1	Control (Mechanical weed control)		
2	Gallant Super	Haloxyfop	1.00
3	Oratio	Carfentrazone	0.03
4	Kontakt 320 SC	Phenmedipham	1.50
5	Lentagran WP	Pyridate	1.00
6	Targa Super	Quizalofop-P	2.00
7	Boxer	Prosulfocarb	5.00
8	Cato	Rimsulfuron	0.03
9	Bandur	Aclonifen	3.01
10	Cirrus 50 WP	Clomazone	0.30

Five of these herbicides (Haloxyfop, Phenmedipham, Pyridate, Quizalofop & Prosulfocarb) were used during 2006 and 2007, whereas eight (Control, Carfentrazone, Phenmedipham, Pyridate, Quizalofop, Prosulfocarb, Rimsulfuron, Aclonifen & Clomazone) were used as experimental treatments against a manually weeded control. Classification of these herbicides depending on mode of action can be observed in table 10.

Table 10: Classification of the herbicides used during the course of the study

Trade Name	Active Ingredient	Chemical Family	Mode of Action	HRAC /WSSA Group
Gallant Super	Haloxyfop-R-methyl	Aryloxyphenoxy-Propionate ‚FOPs'	Inhibition of acetyl CoA carboxylase (ACCase)	A/1
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

HRAC: Herbicide Resistance Action Committee
WSSA: Weed Science Society of America

2.3.4 Study Parameters

Plant height (cm) was measured before the harvest of the crop. Height was recorded from the ground level to the tip of the longest leaf with the help of meter rod. The plant height was recorded for 10 plants from the two middle rows (1, 3, 5, 7 and 9 plants from one row and 2, 4, 6, 8 and 10 plants from the second row) of the plots and the average was worked out. Two middle rows of the plots were counted and plant population in plants m^{-2} was calculated. The data regarding plant cover (%) were collected by visual observation depending on the ground area covered by the artichoke leaves. The data were expressed in percent. Green and yellow leaves per plant were calculated by counting total number of green and yellow leaves from all plants in the two middle rows and average was worked out. Fresh leaf yield (t ha^{-1}) was calculated by harvesting all plants from the two middle rows of all plots and the weight was recorded in Kg. The recorded weight was then converted to t ha^{-1} for final manipulation of data. Two samples of fresh leaves were taken for Dry matter estimation from each plot. The leaf samples were oven dried at 105 °C for a constant dry weight. Percent dry matter was recorded by using the following formula and then average was worked out.

$$\text{Dry Matter (\%)} = \frac{\text{Weight of dried sample (g)}}{\text{Weight of fresh sample (g)}} \times 100$$

Dry matter (t ha^{-1}) was calculated as under;

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

Chlorophyll fluorescence data were recorded by pulse amplitude modulation (PAM) technique. For the field measurements a portable chlorophyll fluorometer, Mini PAM (WALZ) was used. 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. Mini PAM uses the following principle for measuring chlorophyll fluorescence.

(Fm- Ft)/ Fm

Where, Fm= Maximum fluorescence

Ft= Ground/ Zero Fluorescence

Chlorophyll fluorescence data like photosynthetic yield (µ mol CO_2 m^{-2} s^{-1}) and electron transport rate (µ mol m^{-2} s^{-1}) are recorded automatically in the Mini-PAM which were 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 1DAA (days after application of herbicides), 1 WAA (weeks after application of herbicides), 2 WAA, 3 WAA and 4 WAA. Light adapted plants measurements were made under direct sunlight where as for dark-adapted measurements experimental area was covered with dark sheets for four minutes and then measurements were made under this sheet.

2.4 HPLC analysis

The laboratory analyses of the artichoke leaf samples included following steps;

2.4.1 Sample Extraction

Artichoke leaf samples were dried to a maximum temperature of 39 °C for a constant dry weight and then 50 mg finely powdered sample was transferred to a 50 ml volumetric flask. 12.5 ml MeOH (p. a)/ H_2O (bi-distilled) {80/20, v/v} was added to the sample. The sample was then thoroughly mixed and put in the ultra sonic bath for 30 minutes (≤ 39 °C) the flasks were then taken out of it and allowed to cool to the room temperature. Sample was then diluted by adding 12.5 ml H_2O. The contents of the flask were then transferred to a 10 ml test tube (2/3 of tube was filled with solution) and were centrifuged for 15 minutes at 5000-6000 rpm (revolutions per minute). The clear solution was taken out of these tubes by means of syringe and needle and transferred to colored vials using 0.45-µm filters and frozen at –20°C.

2.4.2 HPLC measurements

Hardware/ Software

The system used for the analyses along with its components is described briefly in the form of a table (see table 11). 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.

Table 11: Characterization of the HPLC system used for the polyphenol analyses of artichoke leaves

Part	Description	Company
Degasser	K- 5004 (A 2015)	Knauer
Solvent Organizer	K- 1500 (A 4012)	Knauer
Maxi star pump	K- 1001/VA (A 40301)	Knauer
Dynamic mixing chamber	A 0285	Knauer
Marathon auto sampler	K- 3800 (A 0759)	Knauer
Detector lamp	K-2700	Knauer
DAD (Diode Array Detector)	K-2701	Knauer
Column thermostat	Techlab K-7	Techlab
Pre-column	SecurityGuard cartridges C 18 4 x 3.0 mm	Phenomenex
Column	Synergi 4 µm Hydro-RP 80A 250 x 4.6 mm	Phenomenex
Software	Eurochrom® for windows (V 3.05)	Knauer

Eluents

The eluents used for carrying the sample included Phosphoric acid {H_3PO_4 (0.5 %)}, Acetonitrile {CH_3CN (HPLC grade)} and Water {H_2O (HPLC grade)}. H_3PO_4 (85 %) that was diluted to 0.5 % was bought from the company 'Merck', CH_3CN from the company 'Carl ROTH', where as H_2O used for the analysis from the company 'Applichem'.

Analysis

The gradient method (all three eluents were mixed during the analyses) with a flow rate of 1.0 ml min^{-1} and a wavelength of 330 nm for a time period of 50 minutes (0-5 min. for conditioning and 35-50 min. for cleaning of column) was used to analyze artichoke samples. Negative peaks and those before 5 minutes and after 33 minutes were not considered and excluded during the final calculation. The system was calibrated with pure substances to set the retention time for different substances. Chlorogenic acid, caffeic acid and cynarin (for caffeoylquinic acids) Luteolin-7-glucoside (for flavonoids) were used as standards and first three of these were diluted in MeOH (p. a)/ H2O (bi-distilled) {40/60, v/v} and the last one was diluted in MeOH in 10 ml volumetric flasks. The contents were mixed thoroughly in ultra sonic bath for 15 minutes. 1.0 ml each from all standards was used to make a stock solution (SS). Three different concentrations i.e. A (1ml SS), B (2 ml SS) & C (4 ml SS) were used for calibration, which were filled in vials and one of these vials was used during the analyses every time as standard. Depending on the amount of the standard detected by HPLC analysis a correction factor was calculated and was multiplied with the amount of the substances quantified for the samples. The factor was calculated by using the following formula;

Correction Factor = (detected concentration) / (actual concentration)

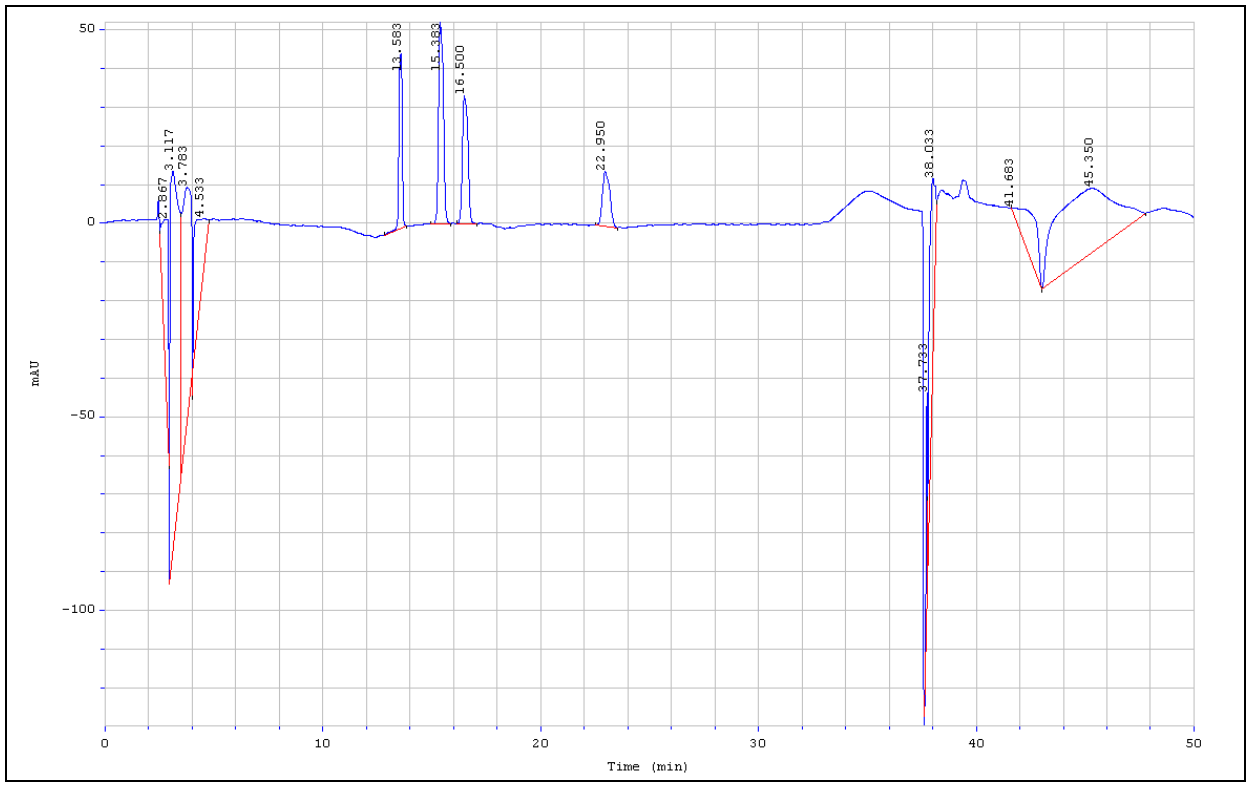


Figure 7: Chromatograph of a pre calibration standard (C).

Chromatograph of a standard solution achieved by analyses with HPLC is shown in figure 7. The chromatograph shows 4 peaks that represent to the reference substances used in this standard solution. The peaks belong to chlorogenic acid (RT: 13.583 min.), caffeic acid (RT: 15.383 min.), cynarin (RT: 16.500 min.) and luteolin-7-glucoside / cynaroside (RT: 22.950 min.), respectively. The negative peaks shown at the beginning of the chromatograph are conditioning peaks and those at the end of the chromatograph are cleaning ones and were ignored for the calculations.

2.4.3 Quantification

Polyphenols (total caffeoylquinic acids and flavonoids) were identified and quantified by high performance liquid chromatography (HPLC) method modified by Brand and Weschta (1991). Chlorogenic acids and isomers of chlorogenic acids were quantified as caffeoylquinic acids and Luteolin-7-glucoside (Cynaroside) and its isomers were quantified as flavonoids in µg ml^{-1}. The concentration was finally converted to percent dry matter by using the following formula;

$$\text{Concentration (\% DM)} = \frac{\text{Conc. (µg ml}^{-1}\text{) x 25 (ml) x 100 (\%)}}{\text{50 (mg) x 1000}}$$

Conc. (µg ml^{-1}): Actual concentration in µg ml^{-1}
25 (ml): volume used for extraction
50 (mg): weight of sample used for extraction
1000: conversion of µg to mg
100 (%): for percent

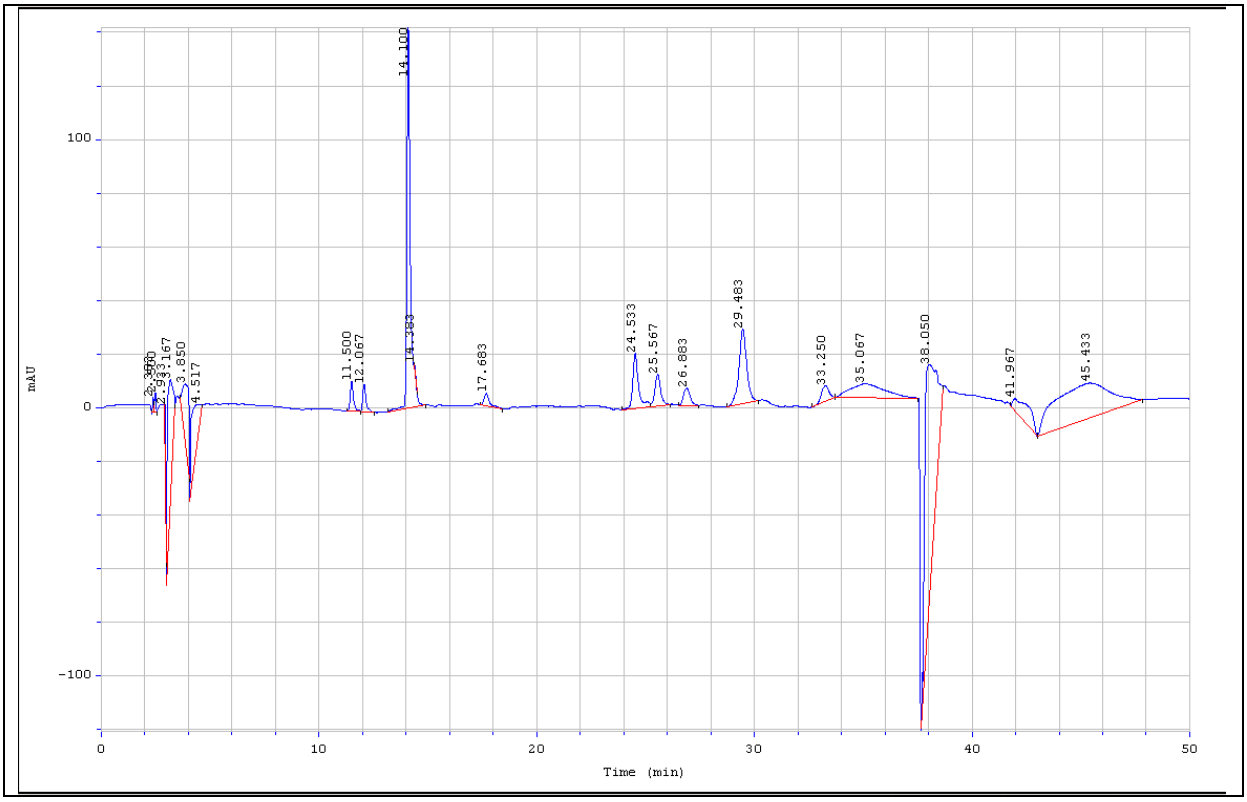


Figure 8: A typical chromatograph of the artichoke leaves for one of the samples obtained during the study, showing different peaks

	A	B	C	D	E	F	G	H	I	J	K	L
1		Ret.time[min]	Start[min]	End[min]	Compound	Amount	Area[mAU*min]	Height[mAU]	Type	Spec.SearchResult	Selectivity	CapacityFactor
2	1	2,3833334	2,3333335	2,4333334	Unknown	-1	0,40166321	5,533606529	BP	Not found	-1	0,191666722
3	2	2,5000002	2,4333334	2,5666668	Unknown	-1	0,56686527	7,10844326	PB	Not found	-1	0,250000119
4	3	2,9333336	2,8833334	2,9833336	Unknown	-1	2,37735271	29,57221031	BB	Not found	-1	0,466666818
5	4	3,166667	3,0000002	3,4000003	Unknown	-1	14,2720871	48,50317383	BB	Not found	-1	0,583333492
6	5	3,8500001	3,6000001	4,0500002	Unknown	-1	7,89776278	21,9504776	BB	Not found	-1	0,925000072
7	6	4,5166669	4,0666671	4,6166668	Unknown	-1	8,51494598	6,610805511	BB	Not found	-1	1,258333445
8	7	11,500001	11,266667	11,916667	Unknown	1,562174258	2,03143597	11,25642776	BP	c	-1	4,750000477
9	8	12,066668	11,916667	12,516667	Unknown	1,302654928	1,69395959	10,09665871	PB	c	-1	5,033333778
10	9	14,100001	13,150001	14,883334	Chlorogenic acid	28,20869794	36,682312	141,8962402	BB	c	-1	6,050000668
11	11	17,683334	17,416668	18,433334	Unknown	-1	1,31273949	4,595174313	BB	Not found	-1	7,841667175
12	12	24,533335	23,916668	25,233335	Cynarosid	11,38015992	6,28738117	20,50994492	BP	f	-1	11,26666737
13	13	25,566668	25,233335	26,116669	Unknown	6,414554825	3,54395294	11,65218449	PB	f	-1	11,78333378
14	14	26,883335	26,516668	27,450003	Unknown	-1	2,23778343	6,351441383	BB	Not found	-1	12,44166756
15	15	29,483335	28,733335	30,166668	Unknown	19,62639331	25,5219679	27,79726601	BB	c	-1	13,74166775
16	16	33,250004	32,666668	33,716667	Unknown	-1	2,34916472	5,881794453	BB	Not found	-1	15,62500191
17	17	35,066669	33,716667	37,533337	Unknown	-1	9,55888367	5,144271374	BB	Not found	-1	16,53333473
18	18	38,050003	37,666668	38,683334	Unknown	-1	53,7051392	87,57126617	BB	Not found	-1	18,02500153
19	19	41,966671	41,76667	43,000004	Unknown	-1	6,00400829	4,363811493	BB	Not found	-1	19,98333549
20	20	45,433334	43,01667	47,850002	Unknown	-1	40,0936127	12,90589714	BB	Not found	-1	21,71666718
21												
22												
23					Chl. Acid	CQA	Cynaroside	Flavonoids				
24					28,20869794	50,69992043	11,3801599	17,79471475				
25												

Figure 9: Peak report of the chromatograph (Fig. 8) showing the concentration of different CQA and Flavonoids

Figures 8 and 9 show the chromatograph and its peak report, respectively. The peak heads are showing the retention times. Peak report is showing the start and end time, name of the compound (standard), amount, area, height and type of the

obtained peaks, whereas column ‘J’ of the report shows the detected compounds after their comparison with the spectral library. Highlighted rows (10 & 12) show chlorogenic acids and cynarosides, respectively. The software calculated the amount of these compounds automatically, whereas the other ones were calculated manually by using unit method. All these amounts were added up as presented in rows 23 and 24 along with the names of the compounds.

2.5 Statistical analysis

Statistical package PIAF (Programm Information Auswertung Feldversuche (Program for Statistical Evaluation of Field Trials) {Dr. Andrea Zenk (modifier) und Volker Michel (conception)} was used for checking the significance of the different treatments used. 5 % probability level was used for studying the difference between different experimental treatments. Least significance difference (LSD) test at 0.05 α was used to compare different treatment means. The standard deviations (SD) showed in figures were calculated by using Microsoft Excel.

3 Results

3.1 Effect of harvest frequency

3.1.1 Field experiment Gross-Gerau 2006

3.1.1.1 Growth and yield parameters

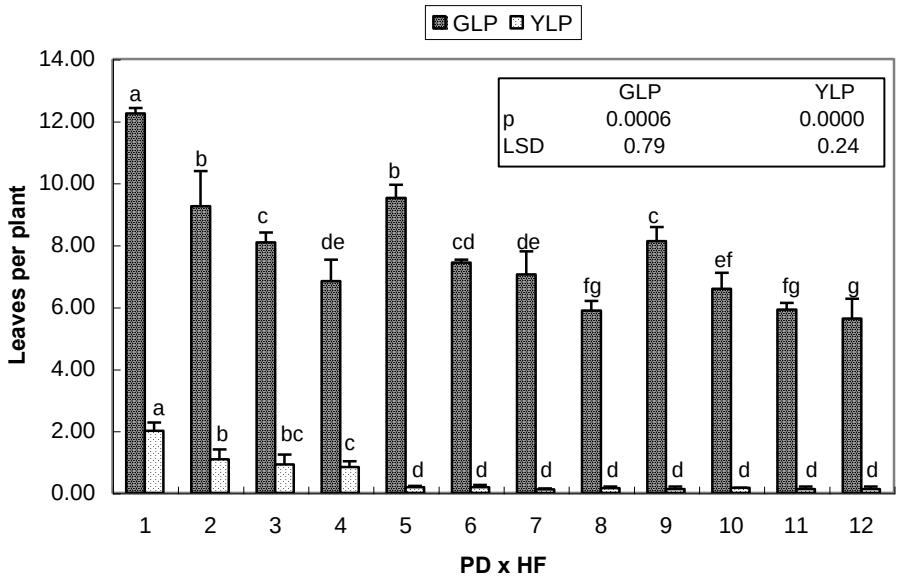
In 2006 the artichoke plants at the time of harvest reached a mean plant height of around 52 cm (HF1, lowest harvest frequency), 39 cm (medium harvest frequency, HF2) and 31 cm (highest harvest frequency, HF3) (table 12). Significant effects of plant density (PD) as well as harvest frequency (HF) but also an interaction between both factors were observed (p value PD x HF = 0.025). Maximum plant height of artichoke was obtained by the combination of 4 plants m^{-2} and lowest harvest frequency (HF1), which was significantly higher than that of other combinations of both factors (table 12). It was followed by 8 plants m^{-2} and 3 cuts, which were statistically at par with that of 12 plants m^{-2} and 3 cuts. Minimum plant height was observed by the combination of 16 plants m^{-2} and highest harvest frequency (HF 3) of 6 cuts, which was similar with that of all other planting densities at higher harvest frequency (6 cuts).

Table 12: Effect of plant density (PD) and harvest frequency (HF) on plant height (cm) in artichoke, Gross Gerau 2006

PD	HF1	HF2	HF3	Mean
4 Pl m^{-2}	57.4 **a**	39.2 **d**	31.0 **e**	**42.5 A**
8 Pl m^{-2}	51.2 **b**	39.9 **d**	31.9 **e**	**40.8 A**
12 Pl m^{-2}	51.6 **b**	39.0 **d**	30.7 **e**	**40.4 A**
16 Pl m^{-2}	46.8 **c**	37.9 **d**	29.0 **e**	**37.9 B**
Mean	**51.7 A**	**38.8 B**	**30.6 C**	
	PD	**HF**	**PD x HF**	
P	0.019	0.000	0.025	
LSD	2.50	2.17	4.33	

Data relating the effects of PD and HF and their interactions on plant cover (%) are to find in appendices A1, A2 and A3 respectively. Interaction of all plant densities with low harvest frequency (3 harvests) led to maximum plant cover, which was statistically higher and different with that of the interactions of medium (5 harvests) and high (6 harvests) harvest frequencies with all the plant densities. High harvest frequency in interaction with all the plant densities produced minimum plant cover with the interaction of lowest plant density (4 pl. m^{-2}) and high harvest frequency showing the thinnest covering of the soil by artichoke leaves within all the interactions.

Interaction of planting density and harvest frequency affected both green and yellow leaves per plant significantly (figure 10). This effect shows that maximum number of leaves per plant (both green and yellow leaves) was found in case of the interaction of lowest plant density and low harvest frequency (4 pl. m^{-2} x 3 cuts), whereas minimum number of leaves per plant was found at highest plant density in combination with high harvest frequency (16 pl. m^{-2} x 6 cuts).



1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

Figure 10: Effect of plant density and harvest frequency on number of green leaves (GLP) and yellow leaves (YLP) per plant in artichoke, Gross Gerau 2006 (T = ±SD)

Interaction of plant density and harvest frequency affected dry matter percentage significantly with maximum dry matter of 11.7 % at 4 pl. m^{-2} x 3 cuts, whereas minimum dry matter of 10.2 % was observed by the combination of 12 pl. m^{-2} x 5 cuts, which was even the results of the other interactions (appendix A4).

Data regarding leaf yield (t ha^{-1}) were collected for all treatments and cuts depending on three methods of harvest frequency, where for each treatment sum of all cuts per season was given and average per cut was calculated (tab. 13). Both parameters PD as well as HF had statistically significant effects on leaf yield (fresh) per treatment (LYT) and leaf yield per cut (LYC). Leaf yield (t ha^{-1}) decreased with an increase in harvest frequency and significant differences in leaf yield were found among all three harvest frequencies used in this experiment.

Table 13: Effect of plant density (PD) and harvest frequency (HF) on leaf yield (FM t ha^{-1}) per treatment (LYT, sum of all harvests per season) and leaf yield per cut (LYC) in artichoke, Gross Gerau 2006

PD	HF1		HF2		HF3		Mean	
	LYT	***LYC***	**LYT**	***LYC***	**LYT**	***LYC***	**LYT**	***LYC***
4 Pl m^{-2}	94.74	*31.58*	76.65	*15.72*	62.52	*10.42*	**77.97 D**	***19.24 C***
8 Pl m^{-2}	101.48	*33.83*	93.48	*18.78*	70.81	*11.80*	**88.59 C**	***21.47 B***
12 Pl m^{-2}	113.63	*37.88*	97.38	*19.49*	81.74	*13.62*	**97.58 A**	***23.66 A***
16 Pl m^{-2}	105.74	*35.25*	103.68	*20.95*	79.65	*13.28*	**96.36 B**	***23.16 AB***
Mean	**103.90 A**	***34.63 A***	**92.80 B**	***18.73 B***	**73.68 C**	***12.28 C***		

LYT: Leaf Yield per Treatment

	PD	**HF**	**PD x HF**
P	0.000	0.000	0.619
LSD	6.94	6.01	NS

LYC: Leaf Yield per Cut

	PD	**HF**	**PD x HF**
p	0.000	0.000	0.581
LSD	1.88	1.63	NS

Dry matter yield of artichoke (t ha^{-1}) was not affected by the interaction PD x HF, although slight differences were observed (appendix 7). Maximum plant density led to maximum dry matter yield for both leaf yield per cut as well as leave yield per treatment that proved to be statistically similar with all other plant densities in the study with the exception of lowest plant density (4 plants m^{-2}), which produced minimum dry matter (fig. 11). Same trend, as that of leaf yield was found regarding the effect of harvest frequency on the dry matter (t ha^{-1}) of artichoke leaves, i.e. an increase in harvest frequency decreased the dry matter. Statistically different dry matter was observed for all harvest frequencies used in the course of the study (figure 12).

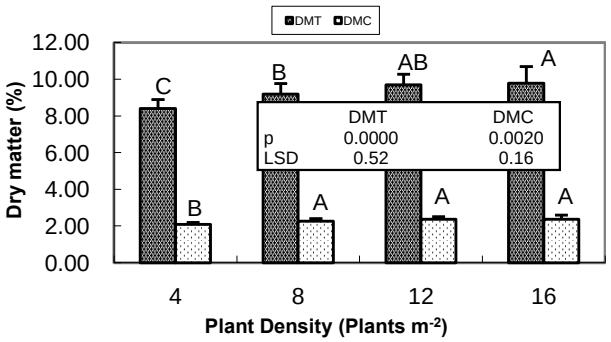


Figure 11: Dry matter (t ha^{-1}) as affected by plant density, Gross Gerau 2006 (T = ±SD)

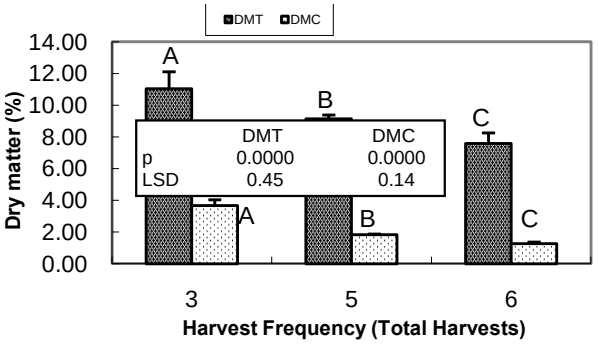


Figure 12: Dry matter (t ha^{-1}) as affected by harvest frequency, Gross Gerau 2006 (T = ±SD)

3.1.1.2 Caffeoylquinic acids and flavonoids

Whole leaves of artichoke plants were characterized by a low level of caffeoylquinic acid concentration of around 1 % (fig. 13 and 14). An increase in plant density caused a decrease of caffeoylquinic acids concentration of artichoke leaves with maximum CQA concentration at 4 pl. m^{-2} and minimum CQA concentration at 16 pl. m^{-2}. Contrary to that flavonoids showed no significant response to different plant densities used in this experiment. An increase in harvest frequency decreased the concentration of caffeoylquinic acids, which were found to be significantly different for all three harvest frequencies used in this trial. Flavonoids also showed an inverse relation with harvest frequency (statistically same for HF1 and HF2).

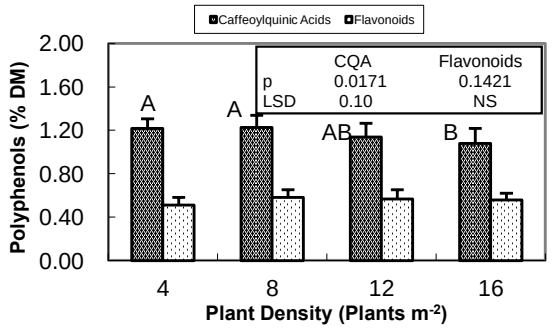


Figure 13: Polyphenols (% DM) in artichoke leaves (whole leaves) as affected by plant density, Gross Gerau 2006 (T = ±SD)

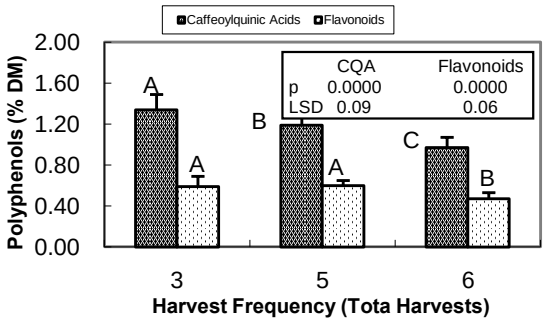


Figure 14: CQA and flavonoid concentration (% DM) in artichoke leaves (whole leaves) as affected by harvest frequency, Gross Gerau 2006 (T = ±SD)

Specific effect of the main factors on polyphenols in artichoke leaf blades is presented in appendices A9 and A10. It was observed that caffeoylquinic acids decreased with an increase in plant density. Maximum values of CQA were found at a level of 4 plants m^{-2} and minimal CQA concentration was observed at 16 plants m^{-2}. Plant density had no significant effect on flavonoids of artichoke leaf blades in 2006. Both caffeoylquinic acids and flavonoids decreased with an increase in harvest frequency with significant differences among all the harvest frequencies used.

Contrary to the concentration of polyphenols in leaf blades, interaction of plant density and harvest frequency resulted in maximum caffeoylquinic acids with 4 pl. m^{-2} x 3 cuts, which proved to be significantly at par with that of 12 pl. m^{-2} x 5 cuts, 8 pl. m^{-2} x 3 cuts, 16 pl. m^{-2} x 6 cuts and 8 pl. m^{-2} x 6 cuts and statistically different with all other interactions studied. Minimum caffeoylquinic acids were found at 12 pl. m^{-2} x 3 cuts which were statistically similar with that of 4 pl. m^{-2} x 5 cuts, 12 pl. m^{-2} x 6 cuts, 8 pl. m^{-2} x 5 cuts, 16 pl. m^{-2} x 5 cuts, 8 pl. m^{-2} x 6 cuts, 12 pl. m^{-2} x 6 cuts and 16 pl. m^{-2} x 6 cuts and different with all other treatments. The interaction of the study factors as well as main factors did not affect both caffeoylquinic acids and flavonoids statistically.

3.1.2 Field experiment Gross-Gerau 2007

3.1.2.1 Growth and yield parameters

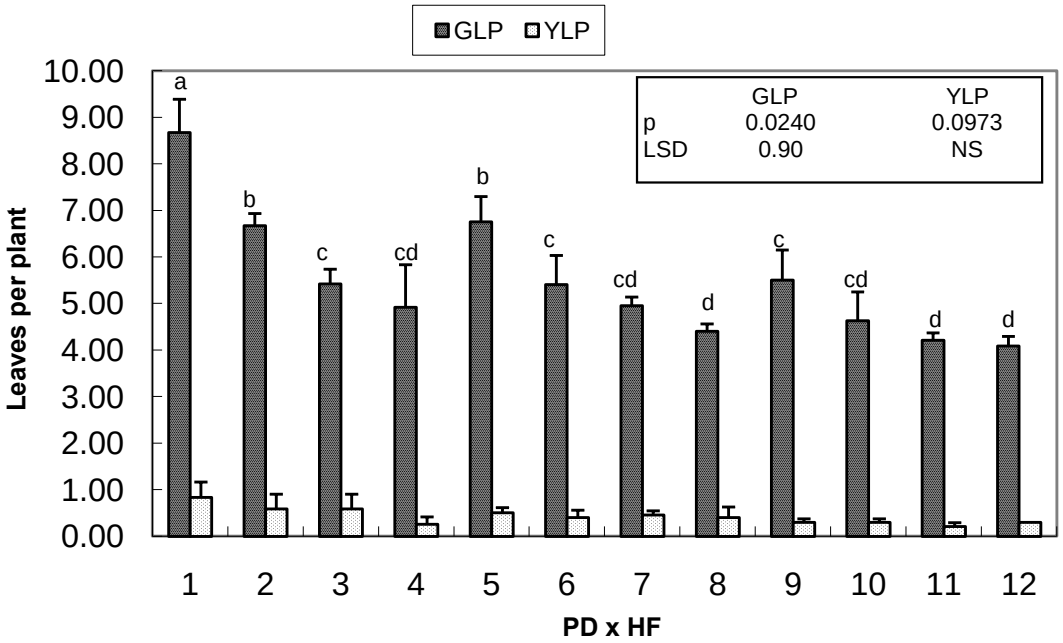
In 2007 the artichoke plants had lower plant height ranging from 22.7 to 44.1 cm at harvest than one year before. Only the harvest frequency had a significant effect on plant height which was highest in HF1 (42.4 cm) and significant lower in HF2 (29.9 cm) and HF3 (24.4 cm) (table 14). Neither plant density (PD) nor an interaction of HF x PD was observed.

Table 14: Effect of plant density (PD) and harvest frequency (HF) on plant height (cm) in artichoke, Gross Gerau 2007

PD	HF1	HF2	HF3	Mean
4 Pl m^{-2}	44.1	29.9	22.7	**32.2**
8 Pl m^{-2}	43.8	29.5	25.3	**32.9**
12 Pl m^{-2}	41.4	30.5	25.1	**32.3**
16 Pl m^{-2}	40.3	29.6	24.6	**31.5**
Mean	**42.4 A**	**29.9 B**	**24.4 C**	
	PD	**HF**	**PD x HF**	
p	0.801	0.000	0.652	
LSD	NS	2.44	NS	

In 2007 there was a significant effect of plant density on plant cover (%) of artichoke (see appendix A16). Maximum plant cover was observed at highest plant density, which reduced with decrease in plant density and reached minimum level at lowest plant density. Plant cover at all these plant densities was significantly different among each other with the exception of highest and higher plant densities that were statistically non significant with each other. However frequent harvest of artichoke plants led to clear effect on plant cover which was reduced by increasing the number of harvests per season (see appendix A 17).

Regarding the parameter leaves per plant, a significant interaction of HF x PD was observed for green leaves per plant, whereas yellow leaves per plant showed a non significant response to this interaction (figure 15). Maximum green leaves per plant were obtained at the combination of lowest plant density and low harvest frequency (4 pl. m^{-2} x 3 cuts) and minimum leaves per plant at an interaction of higher plant density and high harvest frequency (12 pl. m^{-2} x 6 cuts). The number of leaves per plant was generally reduced in the interactions from low harvest frequency towards high harvest frequency including interactions with all the plant densities.



1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

Figure 15: Effect of plant density and harvest frequency on green leaves (GLP) and yellow leaves (YLP) per plant in artichoke in Gross Gerau,2007 (T = ±SD)

Artichoke leaves per plant ranged from 4 to 7 green leaves and around one leaf per plant for different plant densities used during the experimental year (figure 16) and showed a statistically significant response to plant density, which decreased with an increase in plant density, where maximum number of green leaves per plant were found at lowest plant density and minimum ones at highest plant density. Yellow

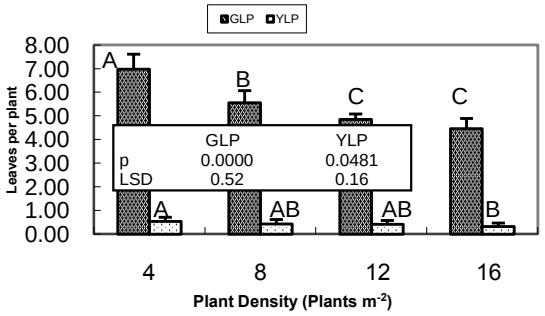


Figure 16: Artichoke leaves per plant of artichoke as affected by plant density, Gross Gerau 2007 (T = ±SD)

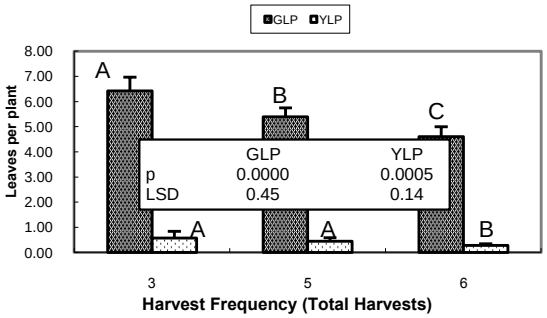


Figure 17: Artichoke leaves per plant as affected by harvest frequency, Gross Gerau 2007 (T = ±SD)

leaves per plant also showed the same trend to the plant density with the exception that maximum yellow leaves were found at lowest plant density and minimum at highest plant density and both the minimum and maximum values were statistically similar with those of lower (8 pl. m^{-2}) and higher (12 pl. m^{-2}) plant densities. Figure 17 explains that green leaves per plant were reduced with an increase in harvest frequency, where these were statistically significant among all three harvest frequencies used during the study. Yellow leaves per plant showed a slightly different response to harvest frequency, where yellow leaves reduced with an increase in harvest frequency but were statistically similar to low and medium harvest frequencies.

Plant density had a significant effect on fresh leaf yield per treatment (LYT) as well as on fresh leaf yield per cut (LYC) (table 15). There was a tendency that maximum leaf yield (per treatment and per cut) was obtained at highest plant density (16 pl. m^{-2}), which was statistically similar with that of higher (12 pl. m^{-2}) and lower (8 pl. m^{-2}) plant density, whereas minimum leaf yield was obtained at lowest plant density (4 pl. m^{-2}). Effect of harvest frequency on leaf yield explains that leaf yield per cut decreased with an increase in harvest frequency and significant difference among all the harvest frequencies was found. Leaf yield per treatment also showed same trend with the exception that leaf yield at high and a medium harvest frequency was statistically same with each other. There was no interaction effect of PD x HF on leaf yield of artichoke plants in the executed field experiment 2007 (table 15).

Table 15: Effect of plant density (PD) and harvest frequency (HF) on leaf yield (FM t ha^{-1}) in artichoke, Gross Gerau 2007

PD	HF1		HF2		HF3		Mean	
	LYT	*LYC*	LYT	*LYC*	LYT	*LYC*	LYT	*LYC*
4 Pl m^{-2}	44.12	*14.71*	41.48	*8.79*	25.11	*4.18*	**36.90 B**	***9.23 B***
8 Pl m^{-2}	54.98	*18.33*	51.23	*10.24*	38.43	*6.40*	**48.21 A**	***11.66 A***
12 Pl m^{-2}	54.58	*18.19*	56.20	*11.38*	44.90	*7.48*	**51.90 A**	***12.35 A***
16 Pl m^{-2}	58.86	*19.62*	51.37	*10.68*	44.25	*7.38*	**51.49 A**	***12.56 A***
Mean	**53.13 A**	***17.71 A***	**50.07 A**	***10.27 B***	**38.17 B**	***6.36 C***		

LYT: Leaf Yield per Treatment

	PD	HF	PD x HF
p	0.000	0.000	0.843
LSD	6.77	5.86	NS

LYC: Leaf Yield per Cut

	PD	HF	PD x HF
p	0.000	0.000	0.705
LSD	1.43	1.24	NS

Maximum dry matter yield per treatment was obtained at highest plant density, which was statistically similar with that of higher and lower plant density and was statistically higher than that of lowest plant density (figure 18). Dry matter per cut increased with an increase in plant density and was lowest at 4 plants m^{-2}, which was statistically lower than that of all other plant densities used. Dry matter obtained at higher plant density was simultaneously similar with that of highest plant density (maximum dry matter yield) and lower plant density. Effect of harvest frequency on dry matter yield of artichoke presented in figure 19 shows that dry matter yield had an inverse relation with harvest frequency during 2007 at Gross Gerau and decreased with an increase in harvest frequency for both dry matter yield per treatment and per cut.

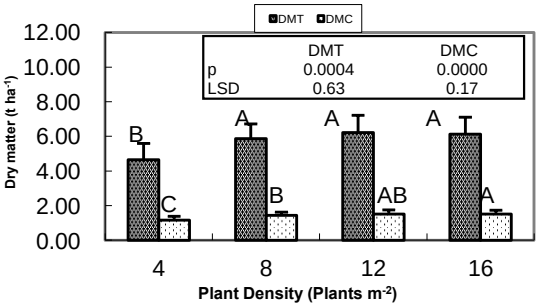


Figure 18: Dry matter (t ha^{-1}) as affected by plant density, Gross Gerau 2007 (T = ±SD)

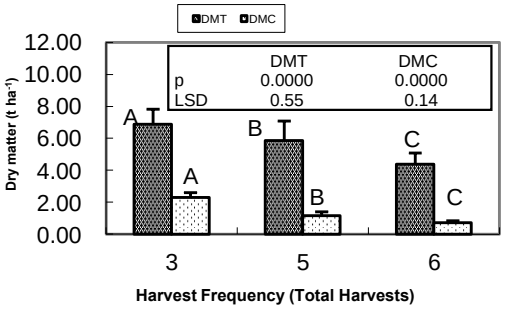


Figure 19: Dry matter (t ha^{-1}) as affected by harvest frequency, Gross Gerau 2007 (T = ±SD)

3.1.2.2 Caffeoylquinic acids and flavonoids

Maximum concentration of caffeoylquinic acids was obtained at lower plant density (8 plants m^{-2}) that was statistically similar with that of lowest plant density (4 plants m^{-2}) and minimum concentration was observed at highest plant density (16 plants m^{-2}) and were statistically similar with that of higher plant density (12 plants m^{-2}) (fig. 20). Harvest frequency and plant density had no significant effect on flavonoids, whereas caffeoylquinic acids too were not affected significantly by harvest frequency (figures 20 & 21) during the year 2007.

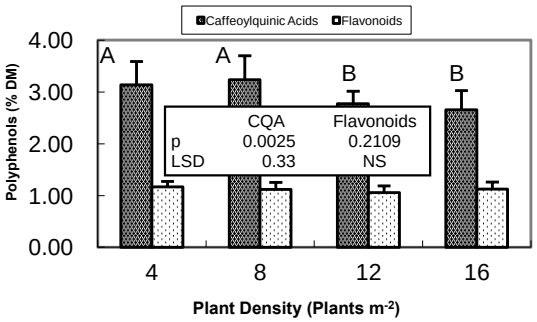


Figure 20: CQA and flavonoids (% DM) in artichoke leaves as affected by plant density in Gross Gerau during 2007 (T = ±SD)

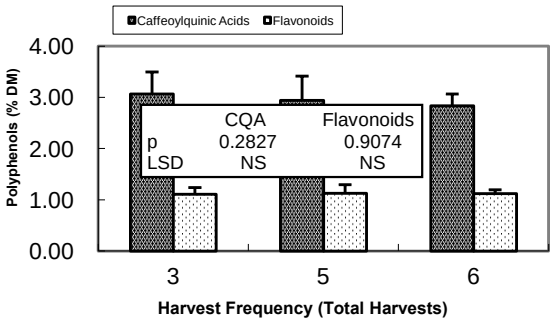


Figure 21: CQA and flavonoids (% DM) in artichoke leaves as affected by harvest frequency in Gross Gerau during 2007 (T = ±SD)

A non significant response of chlorogenic acids and significant one of cynarosides (% DM) in response to the interaction of plant density and harvest frequency is presented in appendix A23. Figure in appendix 23 explains that maximum cynarosides were obtained with an interaction of lower plant density and low harvest frequency (8 pl. m^{-2} x 3 cuts) and minimum ones were found in lowest plant density and medium harvest frequency (4 pl. m^{-2} x 5 cuts). No specific trend of the interaction of studied factors in relation to chlorogenic acids and cynarosides was observed (Appendix A23). Figure A24 demonstrates that maximum chlorogenic acids were obtained in case of lower plant density that was statistically at par with that of lowest plant density. Chlorogenic acids were increased from lowest plant density towards lower plant density and then decreased onwards with minimum chlorogenic acids produced at highest plant density. Cynarosides showed a non significant response to the plant densities used in the study. Appendix A25 depicts the effect of harvest frequency on chlorogenic acids and cynarosides of artichoke during 2007. A close

observation of the figure reveals that maximum chlorogenic acids were obtained at high harvest frequency followed by that of low harvest frequency. Minimum chlorogenic acids can be observed in case of medium harvest frequency. Harvest frequency had no significant influence on cynarosides in artichoke leaves during 2007.

3.1.3 Field experiment Gross-Gerau 2008

3.1.3.1 Growth and yield parameters

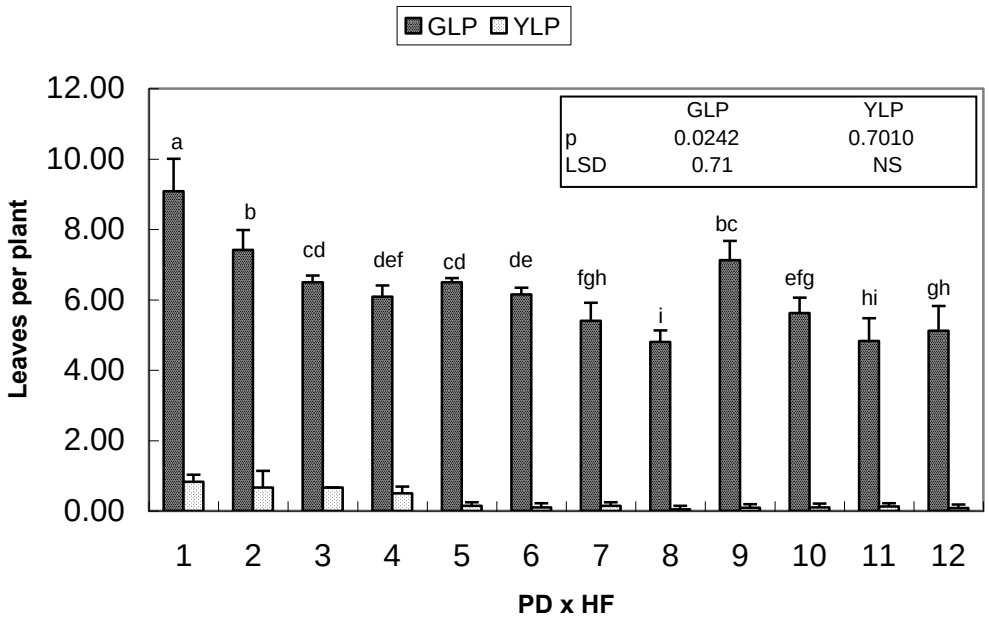
In 2008 plant height of artichoke was influenced only by harvest frequency (table 16). It can be stated that plant height reduced from the lowest harvest frequency (HF1) towards the higher harvest frequency (HF2 and HF3). Neither an effect of plant density nor an interaction of PD x HF was observed.

Table 16: Effect of plant density (PD) and harvest frequency (HF) on plant height (cm) in artichoke, Gross Gerau 2008

PD	HF1	HF2	HF3	Mean
4 Pl m^{-2}	43.0	25.6	21.5	**30.0**
8 Pl m^{-2}	42.8	29.5	20.9	**31.1**
12 Pl m^{-2}	43.7	28.4	20.2	**30.8**
16 Pl m^{-2}	40.5	27.0	21.4	**29.6**
Mean	**42.5 A**	**27.6 B**	**21.0 C**	
	PD	**HF**	**PD x HF**	
p	0.281	0.000	0.108	
LSD	NS	1.46	NS	

Maximum plant cover during 2008 was observed against highest plant density, which was slightly higher but statistically similar with that of higher and lower plant densities (Appendix A27). Lowest plant density showed minimum and statistically lower plant cover when compared with that of other plant densities used in the course of the study. Harvest frequency also affected plant cover significantly that can be observed in appendix A28, which shows an inverse relation between harvest frequency and plant cover, where plant cover obtained at all harvest frequencies were statistically different among each other.

Maximum leaves per plant (both green and yellow leaves) were obtained in case of the interaction of low harvest frequency and lowest plant density (fig. 22). Green leaves per plant in this case were statistically higher and different with that of all other interactions of the study factors, whereas yellow leaves observed for this interaction were statistically similar with that of higher and lower plant densities interaction with low harvest frequency. Interaction of medium harvest frequency with highest plant density showed minimum number of green and yellow leaves of artichoke during 2008. Yellow leaves per plant for the interactions of both medium and high harvest frequency in interaction with all plant densities were found statistically at par with each other. The interactions of all harvest frequencies showed a declining trend of green leaves per plant with increasing plant densities.



1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

Figure 22: Effect of plant density and harvest frequency on green leaves (GLP) and yellow leaves (YLP) per plant in artichoke, Gross Gerau 2008 (T = ±SD)

Green leaves per plant showed a statistically significant response to the plant densities used in the research study, where maximum green leaves per plant were found at lowest plant density and decreased inversely with increasing plant density (Figure 23). Different plant densities had no significant effect on number of yellow leaves per plant. Significant effect of harvest frequency on green and yellow leaves per plant presented in figure 24 explains that low harvest frequency produced maximum number of both green and yellow leaves per plant that were statistically higher than that of medium and high harvest frequency, which were statistically same with each other.

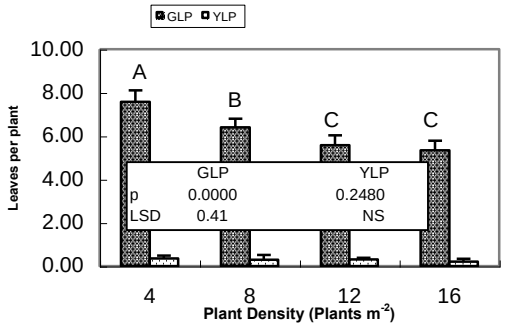


Figure 23: Leaves per plant as affected by plant density, Gross Gerau 2008 (T = ±SD)

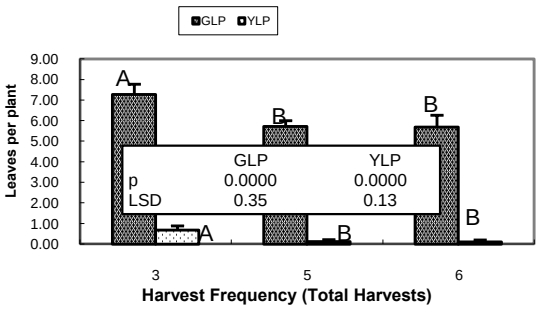


Figure 24: Leaves per plant as affected by harvest frequency, Gross Gerau 2008 (T = ±SD)

Maximum dry matter percentage was obtained in an interaction of low harvest frequency and highest plant density that was slightly higher but statistically similar with that of interaction of lowest plant density and low and medium harvest frequencies (Appendix A29). Dry matter studied at all other interactions of the study

factors was statistically same among each other and lower than that of above mentioned interactions.

Data relating leaf yield (FM t ha^{-1}) of artichoke arranged in Table 17 shows the specific and interaction effect of the study factors on leaf yield of artichoke. A close observation of the said table reveals that interaction of low and medium harvest frequency with lower, higher and highest plant density produced maximum leaf yield per treatment. Minimum leaf yield per treatment was observed in case of the interaction of all plant densities with high harvest frequency when compared with that of interactions of other harvest frequencies. Lowest plant density showed minimum leaf yield per cut, which was statistically lower than that of other plant densities used in the study. All other plant densities used showed slightly different but statistically same leaf yield (table 17). Significant effect of harvest frequency on leaf yield of artichoke shown in table reveals that leaf yield per cut had an inverse relationship with harvest frequency.

Table 17: Effect of plant density and harvest frequency on leaf yield (FM t ha^{-1}) in artichoke, Gross Gerau 2008

PD/ HF	HF1		HF2		HF3		Mean	
	LYT	*LYC*	LYT	*LYC*	LYT	*LYC*	LYT	*LYC*
4 Pl m^{-2}	43.6 **bc**	*14.5*	29.5 **de**	*6.5*	27.3 **e**	*4.5*	**33.5 B**	***8.5 B***
8 Pl m^{-2}	55.9 **a**	*18.6*	52.4 **a**	*10.5*	36.2 **cd**	*6.0*	**48.2 A**	***11.7***
12 Pl m^{-2}	58.1 **a**	*19.4*	52.7 **a**	*10.5*	38.5 **c**	*6.4*	**49.8 A**	***12.1 A***
16 Pl m^{-2}	49.6 **ab**	*16.6*	54.4 **a**	*11.2*	42.4 **bc**	*7.1*	**48.8 A**	***11.6 A***
Mean	**51.8 A**	***17.3 A***	**47.3 B**	***9.7 B***	**36.1 C**	***6.0 C***		

LYT: Leaf Yield per Treatment **LYC:** Leaf Yield per Cut

	PD	HF	PD x HF		PD	HF	PD x HF
p	0.00	0.000	0.050	**p**	0.004	0.000	0.568
LSD	5.05	4.38	8.75	**LSD**	1.98	1.71	NS

Specific effect of plant density and harvest frequency on dry matter yield (t ha^{-1}) of artichoke during 2008 is presented in figures 25 and 26, respectively. All the plant densities except lowest plant density showed statistically similar dry matter yield among each other, which was statistically higher than that of lowest plant density, which depicted minimum dry matter. It was true for both dry matter yields per treatment and per cut (figure 25). Figure 26 explains that harvest frequency had an inverse relation with dry matter, which decreased with an increase in harvest

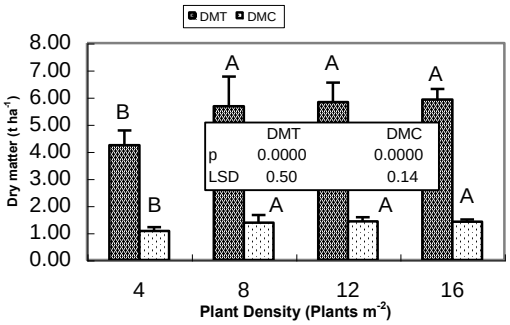


Figure 25: Dry matter (t ha^{-1}) as affected by plant density, Gross Gerau 2008 (T = ±SD)

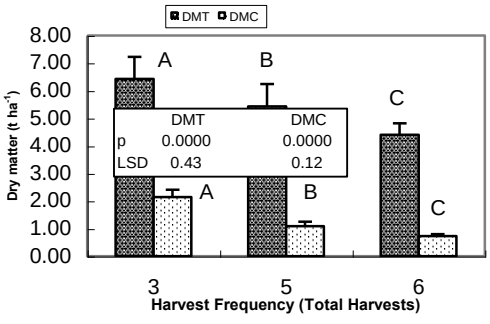
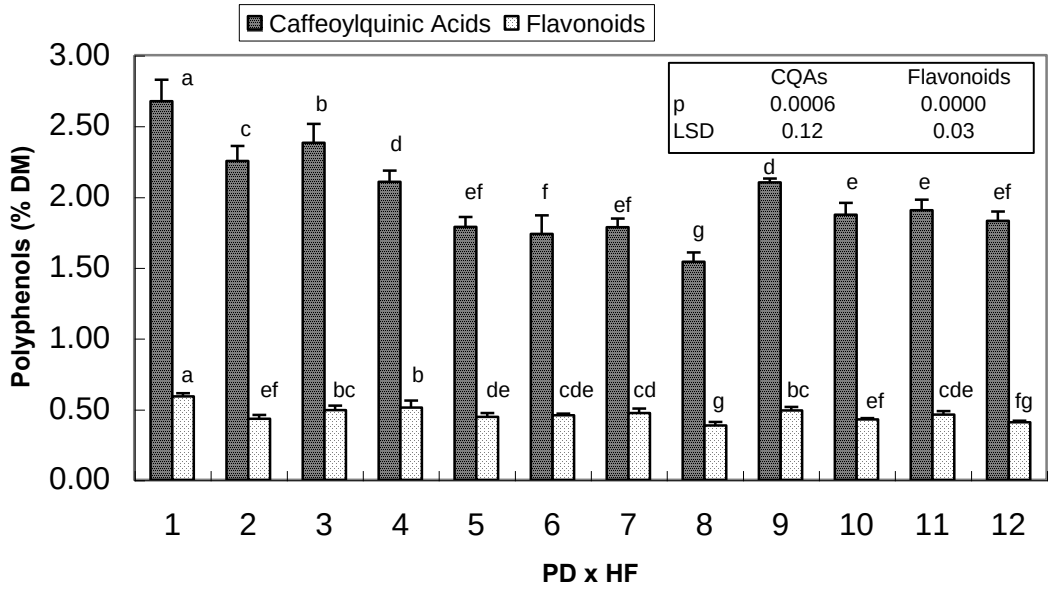


Figure 26: Dry matter (t ha^{-1}) as affected by harvest frequency, Gross Gerau 2008 (T = ±SD)

frequency. All the dry matters recorded at different harvest frequencies were statistically significant with one another.

3.1.3.2 Caffeoylquinic acids and flavonoids

Statistically profound effect of the PD x HF on total polyphenols (caffeoylquinic acids and flavonoids) in artichoke presented in figure 27 explain that maximum caffeoylquinic acids and flavonoids were synthesized by an interaction of lowest plant density and low harvest frequency. In contrast to that interaction of highest plant density and medium harvest frequency led to minimum polyphenol contents. Other interactions of the studied factors showed a mixed response to polyphenols where the interactions of all plant densities with low harvest frequency showed comparatively higher contents of polyphenols. Appendices A36 and A37 depict the main effect of study factors on polyphenol contents of artichoke leaves during 2008 in Gross Gerau.



1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

Figure 27: Effect of plant density and harvest frequency on caffeoylquinic acids and flavonoids (% DM) in artichoke leaves in Gross Gerau during 2008 (T = ±SD)

Interaction of lowest plant density and low harvest frequency led to maximum concentration of chlorogenic acids and cynarosides (appendix A33) during the last year of experimentation. Minimum concentration of these compounds was observed against an interaction of highest plant density and medium harvest frequency. All other interactions studied showed a mixed response to the study factors. Individual effect of plant density and harvest frequency on the contents of chlorogenic acids and cynarosides is shown in appendices A34 and A35, respectively.

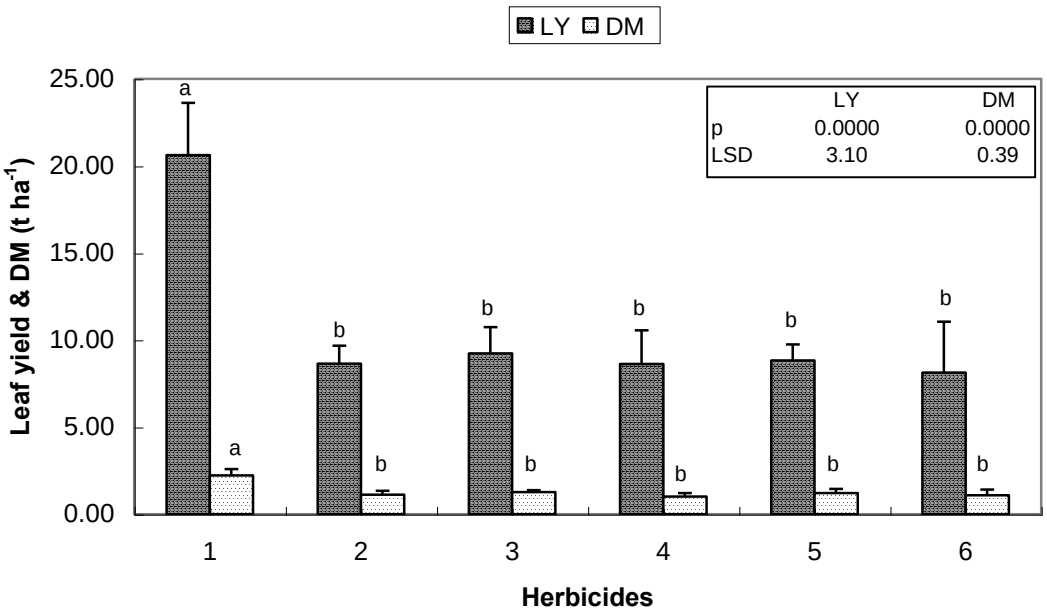
3.2 Effect of herbicides

Effect of different herbicides on growth, yield and quality parameters of artichoke leaves was studied in the field experiments held at Giessen, where for the first two years (2006 & 2007) five different post emergence herbicides and during the third year (2008) eight different post emergence herbicides were used against a manually weeded control. Data on study parameters was collected for two growth periods. Data relating toxicity and photosynthetic parameters were collected periodically during the growth phases, whereas leaf yield data were recorded at both harvests and polyphenols were identified and quantified afterwards in laboratory.

3.2.1 Field experiment Giessen 2006

3.2.1.1 Leaf yield

Maximum leaf yield (fresh and dry matter) was obtained in control, which was statistically higher than that of all the herbicide treatments used during first growth phase 2006 in Giessen (figure 28). Both leaf yield and dry matter produced by the application of different herbicides showed marked differences among each other, but these differences were statistically similar with each other.



1: Control	**2:** Haloxyfop	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P	**6:** Prosulfocarb

Figure 28: Effect of herbicides on leaf yield (FM and DM t ha^{-1}) of artichoke in Giessen during 1st growth phase 2006 (T = ±SD)

Mechanically weeded control resulted in maximum leaf yield (fresh and dry matter) that was statistically higher than that of all other treatments where different post emergence herbicides were applied to artichoke (table 18). Application of Pyridate produced minimum leaf and dry matter yield, which was found significantly different and lower than that of all other treatments used for the research study.

Table 18: Effect of herbicides on leaf yield (FM and DM t ha^{-1}) of artichoke, 2nd growth phase 2006 Giessen

Herbicidal Treatments	Leaf yield (t ha^{-1})	Dry Matter (t ha^{-1})
Control	36.33 **a**	4.29 **a**
Haloxyfop	25.14 **bc**	3.06 **bc**
Phenmedipham	26.83 **b**	3.24 **b**
Pyridate	18.46 **d**	2.09 **d**
Quizalofop-P	22.81 **bcd**	2.64 **cd**
Prosulfocarb	22.55 **cd**	2.81 **c**
p	**0.0000**	**0.0000**
LSD	***4.63***	***0.52***

Percent dry matter in relation to the herbicides used during the course of study at first harvest during 2006 in Giessen presented graphically in appendix A38 reveals that application of Phenmedipham produced maximum dry matter percentage in artichoke leaves that was statistically similar with that of Quizalofop-P, Prosulfocarb and Haloxyfop. Minimum dry matter percentage was observed when no herbicide was applied to artichoke and was statistically lower than that of all other herbicidal treatments. Contrary to the first growth phase the herbicidal treatments had no significant effect on dry matter percentage of the artichoke leaves at second harvest, although slight differences among different treatments were observed (appendix A39).

3.2.1.2 Plant stand parameters

Herbicide application led to different plant height of artichoke plants at the end of the first growth phase but no effect was observed in the second growth phase (table 19). During first growth phase maximum plant height was observed in control, which was statistically different and higher than that of all other herbicide treatments. Plant height attained by the treatments of the herbicides showed slight differences among each other, but these differences were significantly at par with each other. During second growth phase different herbicides had non significant effect on height of artichoke, although different treatments produced slightly different plant heights.

Effect of the post emergence herbicides on the plant population of artichoke at first harvest during 2006 presented in appendix A40 shows that control produced maximum plants per unit area, which were found nearly similar with that of Pyridate, Phenmedipham, Quizalofop-P and Haloxyfop. Minimum plant population was observed after application of Prosulfocarb, which was similar with that of Quizalofop-P and Haloxyfop. Appendix A41 shows the effect of herbicides on plant population of

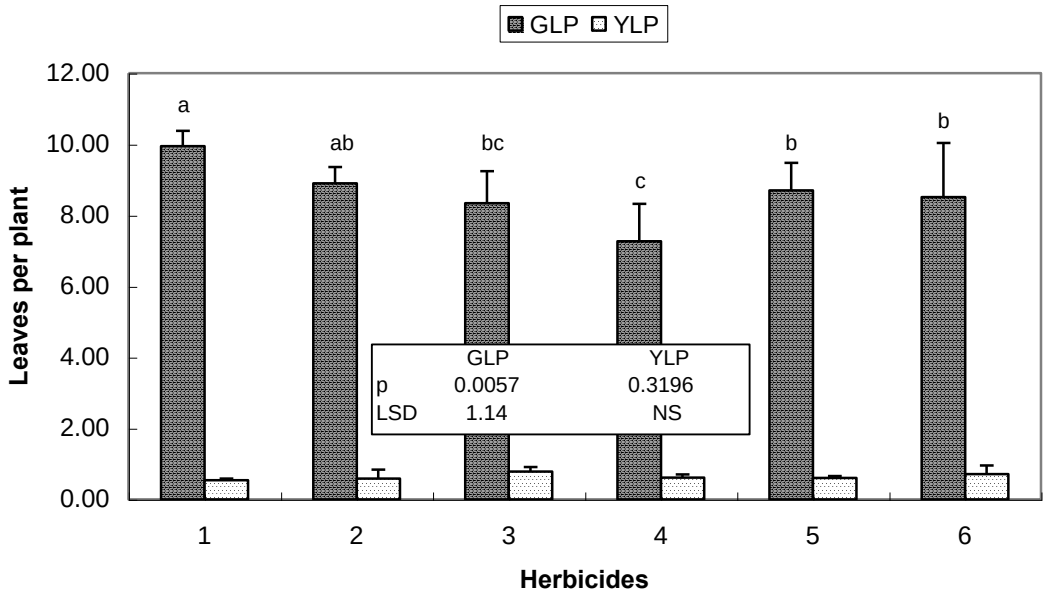
Table 19: Effect of herbicides on plant height (cm) of artichoke, Giessen 2006

Herbicidal Treatments	1st Growth Phase	2nd Growth Phase
Control	80.5 **a**	84.6
Haloxyfop	56.3 **b**	93.4
Phenmedipham	58.6 **b**	88.7
Pyridate	60.7 **b**	85.5
Quizalofop-P	54.3 **b**	94.0
Prosulfocarb	56.5 **b**	89.2
p	**0.000**	**0.481**
LSD	***6.87***	***NS***

artichoke during second growth phase, 2006. Maximum plant population per unit area in this case was obtained in case of the application of Pyridate and minimum one was obtained with the application of Prosulfocarb. Plant populations obtained at other herbicidal treatments were nearly similar with either the highest or the lowest value (appendix A41).

Figure arranged in appendix A42 shows the effect of herbicides on plant cover (%) of artichoke at first harvest in 2006 in Giessen. The data show that maximum plant cover was obtained in control that was statistically different with that of all other treatments. Plant cover obtained at other treatments showed slightly different values but these were statistically same with each other. Percent plant cover, at second harvest during 2006 arranged in the form of figures in appendix A43, explains that maximum percent plant cover was obtained in case of control and minimum where Pyridate was applied. All other herbicides applied to the artichoke produced the plant cover that although were slightly different but were statistically similar among each other.

The artichoke plants developed around 7 to 10 leaves per plant which differs between the treatments (figure 29). Maximum number of green leaves per plant was obtained in the control where no herbicides were applied and these were statistically different with all other herbicidal treatments with the exception of the application of Haloxyfop.



1: Control **2:** Haloxyfop **3:** Phenmedipham **4:** Pyridate **5:** Quizalofop-P **6:** Prosulfocarb

Figure 29: Effect of herbicides on leaves per plant of artichoke, 1st growth phase 2006 Giessen (T = ±SD)

Minimum number of green leaves per plant was observed by the application of Pyridate (no. 4) that was statistically at par with that of Phenmedipham and different with that of all other treatments. Yellow leaves per plant were not affected significantly by the application of different herbicides, although slightly different yellow leaves per plant were observed in case of different treatments used in the study. Contrary to the first growth phase in the second period of plant development no

effects of herbicides on leaf number were observed neither green leaves nor yellow leaves (table 20).

Table 20: Effect of herbicides on leaves per plant of artichoke in Giessen, 2nd growth phase 2006

Herbicidal Treatments	Green Leaves per Plant	Yellow Leaves per Plant
Control	7.11	0.50
Haloxyfop	7.92	0.57
Phenmedipham	7.46	0.67
Pyridate	6.72	0.28
Quizalofop-P	7.27	0.38
Prosulfocarb	7.82	0.47
p	**0.5321**	**0.3528**
LSD	***NS***	***NS***

3.2.1.3 Chlorophyll fluorescence

It was observed that Pyridate affected chlorophyll fluorescence (under direct sunlight) at 1 DAA adversely, whereas minimum chlorophyll fluorescence was obtained followed by application of Phenmedipham (figure 30). Spraying of other herbicides showed similar chlorophyll fluorescence values when compared with that of control. The same trend was observed at 2 DAA. The artichoke leaves recovered against this stress and at 1 WAA, chlorophyll fluorescence values obtained by the application of Pyridate and Phenmedipham were comparatively closer to the control, although these were statistically lower than that of control and other herbicides applied. Chlorophyll fluorescence values obtained at 3 WAA and 4 WAA were statistically at par with each other, and with that of control too. It was observed that two herbicides

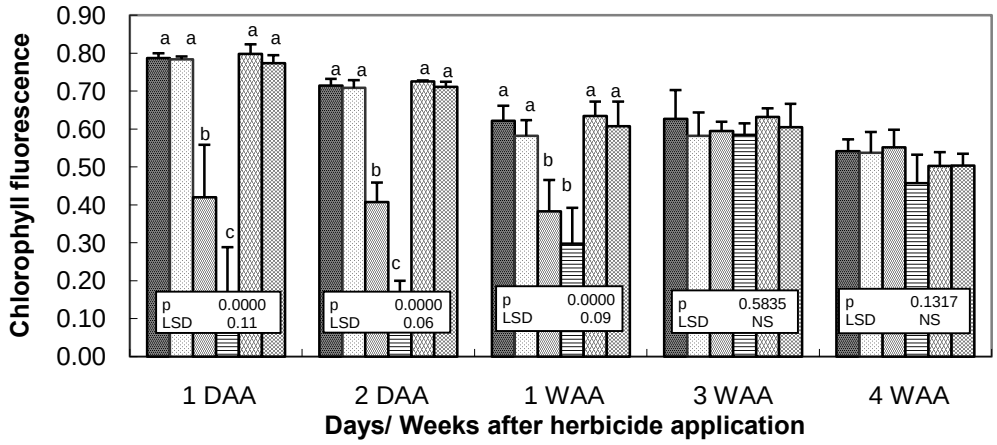


Figure 30: Effect of herbicides on photosynthetic yield (µmol CO_2 m^{-2} sec^{-1}) of artichoke leaves under light adapted conditions in Giessen, 1st growth phase 2006 (T = ±SD)

(Phenmedipham and Pyridate) had significantly effects on chlorophyll fluorescence of artichoke leaves (figure 30).

The application of Pyridate at 1 DAA affected chlorophyll fluorescence (dark adapted) most adversely, where minimum chlorophyll fluorescence value was obtained (figure 31). A less adverse effect was found with Phenmedipham which showed a higher chlorophyll fluorescence value when compared with that of Pyridate but statistically lower value when compared with that of control, which showed statistically same chlorophyll fluorescence values with that of Haloxyfop, Quizalofop-P and Prosulfocarb. At 2 DAA of herbicides same trend was observed, where affect of Pyridate was worst followed by that of Phenmedipham. The data recorded at 1 WAA shows that worst affect of Pyridate was counteracted by artichoke to a certain extent although it was statistically lower than that of all other application treatments, which were statistically same with each other. Chlorophyll fluorescence data recorded at 3 WAA showed significant differences among the treatments studied. It is quite obvious from the figure 31 that, although, application of Pyridate produced statistically lower chlorophyll fluorescence but this was very close to that of control giving a hint of the recovery of artichoke against the adverse effect of the herbicide. Chlorophyll fluorescence data recorded at 4 WAA of the herbicides showed a non significant difference of the values among different treatments used during the course of the study and explains that perhaps the crop recovered against the adverse effects imposed by a few herbicides including Pyridate and Phenmedipham.

Effect of herbicidal treatments on the electron transport rate (µ mol m^{-2} sec^{-1}) under direct sunlight conditions is presented in figure 32. By the observation of figure 32, it is clear that Pyridate had most adverse affect on the ETR of artichoke leaves at 1 DAA which was followed by a less adverse affect imposed by Phenmedipham and both these values were statistically different with one another and also with all other treatments, which were statistically similar with each other. Same trend can be

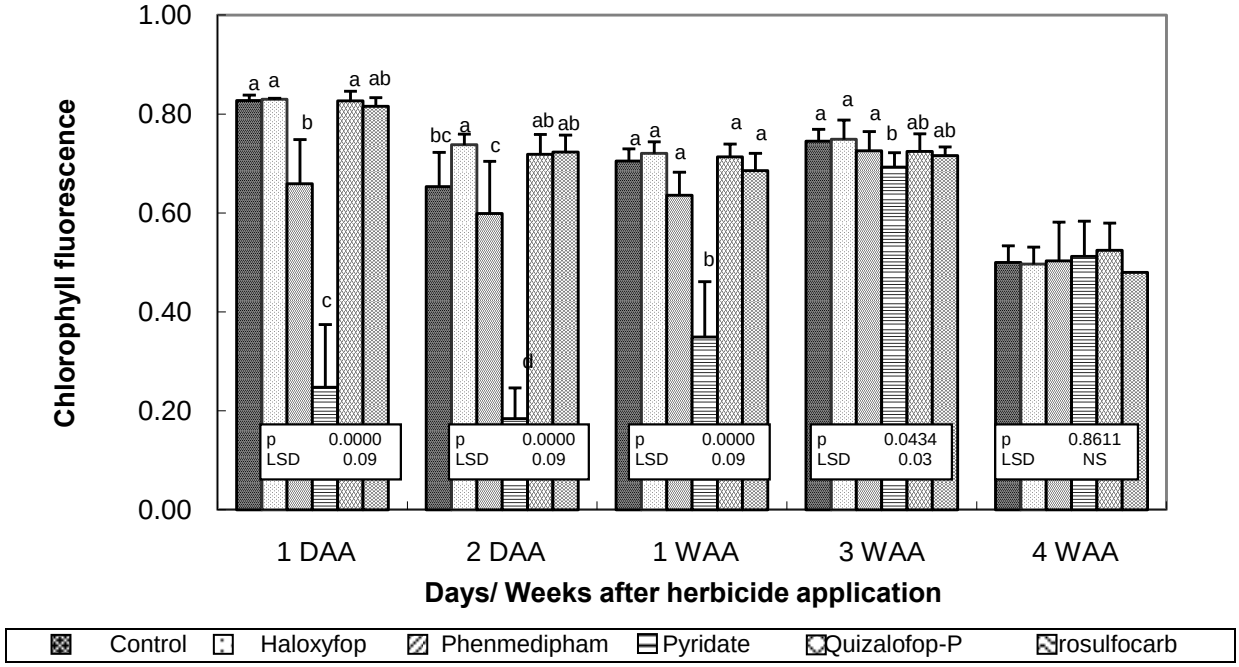


Figure 31: Effect of herbicides on photosynthetic yield (µmol CO_2 m^{-2} sec^{-1}) of artichoke leaves under dark adapted conditions in Giessen, 1st growth phase 2006 (T = ±SD)

observed at 2 DAA, whereas a completely different response of ETR was observed in case of the data recorded at 1 WAA, where Quizalofop-P showed most adverse effect that was statistically at par with that of Phenmedipham. ETR recoded at 3 and 4 WAA showed non-significant effect of the herbicides on ETR in artichoke.

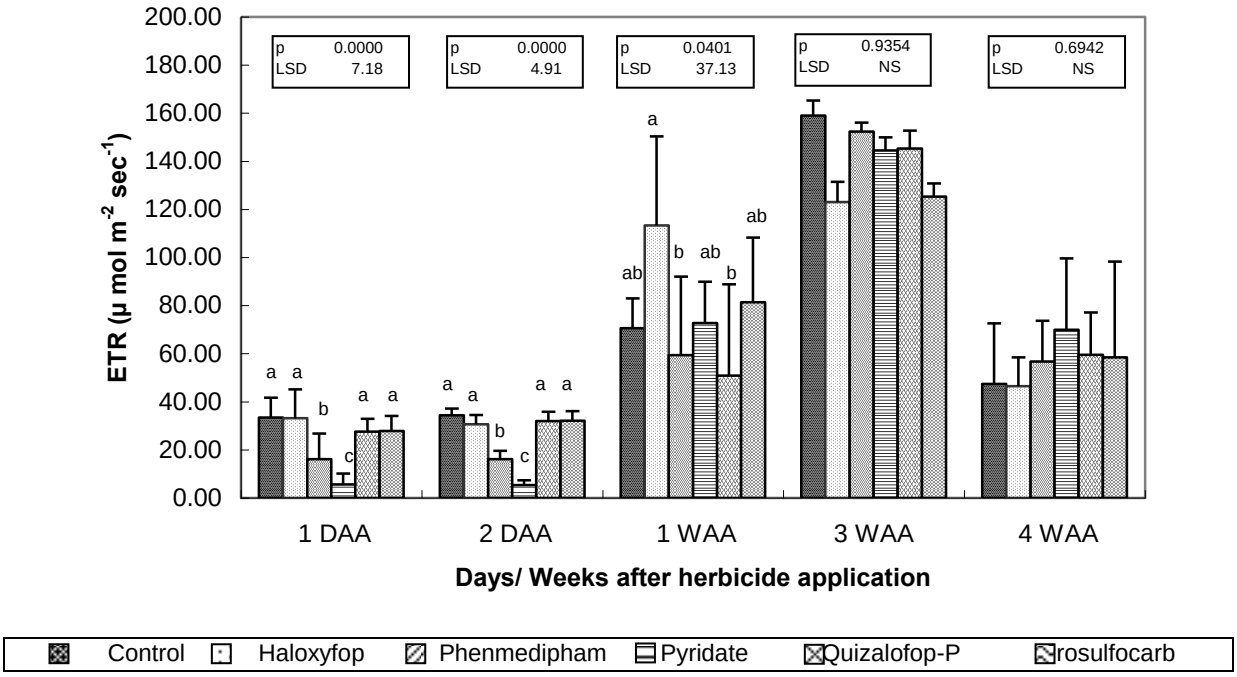


Figure 32: Effect of herbicides on electron transport rate (µmol m^{-2} sec^{-1}) of artichoke leaves under light adapted conditions in Giessen, 1st growth phase 2006 (T = ±SD)

Pyridate imposed the most severe effect at 1 DAA followed by that of Quizalofop-P and Phenmedipham which were significantly same with one another (figure 33). Maximum chlorophyll fluorescence in this case was observed where Haloxyfop was applied that was statistically same with that of control. The application of Prosulfocarb also induced lower chlorophyll fluorescence value in comparison with the control, although it was found to be statistically at par with that of control. The crop recovered against the stress imposed as is clear from the figure at 2 DAA, where artichoke recovered against all the herbicides with the exception of Pyridate and Phenmedipham, which showed most and less adverse affect, respectively. Same trend was observed at 1 WAA, where artichoke showed a worst response to Pyridate and produced minimum chlorophyll fluorescence.

Statistically significant effect of the herbicides on chlorophyll fluorescence under dark adapted conditions at 1 DAA, 2 DAA, 1 WAA and 3 WAA and a non significant effect at 4 WAA is depicted in figure 34. At 1 and 2 DAA, minimum chlorophyll fluorescence was observed in case of the application of Pyridate, which was statistically different and lower than that of all other treatments under study. Worst effect of Pyridate was observed at 1 WAA followed by a less adverse effect of Phenmedipham which were statistically different with each other and with all other treatments used in the study. A non significant effect of treatments studied at 3 WAA was observed with minimum chlorophyll fluorescence value obtained by the application of Phenmedipham.

Effect of different herbicidal treatments on the electron transport rate of artichoke under direct sunlight in 2nd growth phase, 2006 presented in figure 35 shows a non significant effect of the herbicides after one day of application although there were clear differences among the ETR values obtained in response to different herbicidal treatments, whereas it proved to be statistically significant effect at 2 DAA and 1

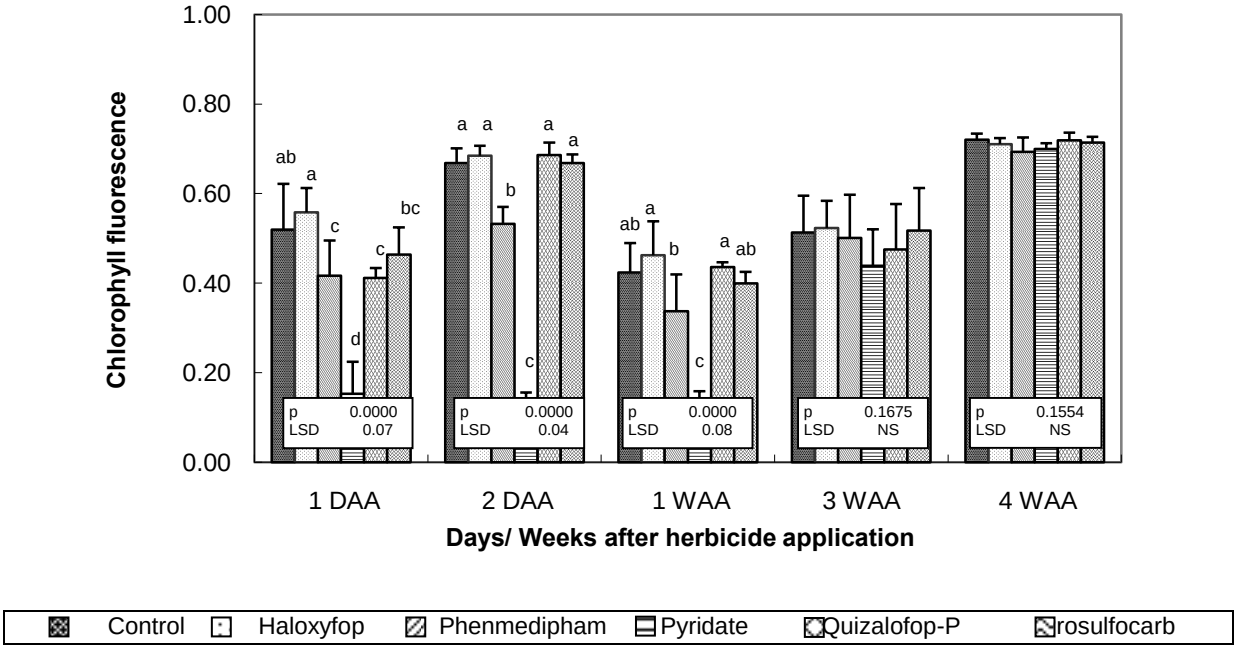


Figure 33: Effect of herbicides on chlorophyll fluorescence of artichoke leaves under light adapted conditions in Giessen, 2nd growth phase 2006 (T = ±SD)

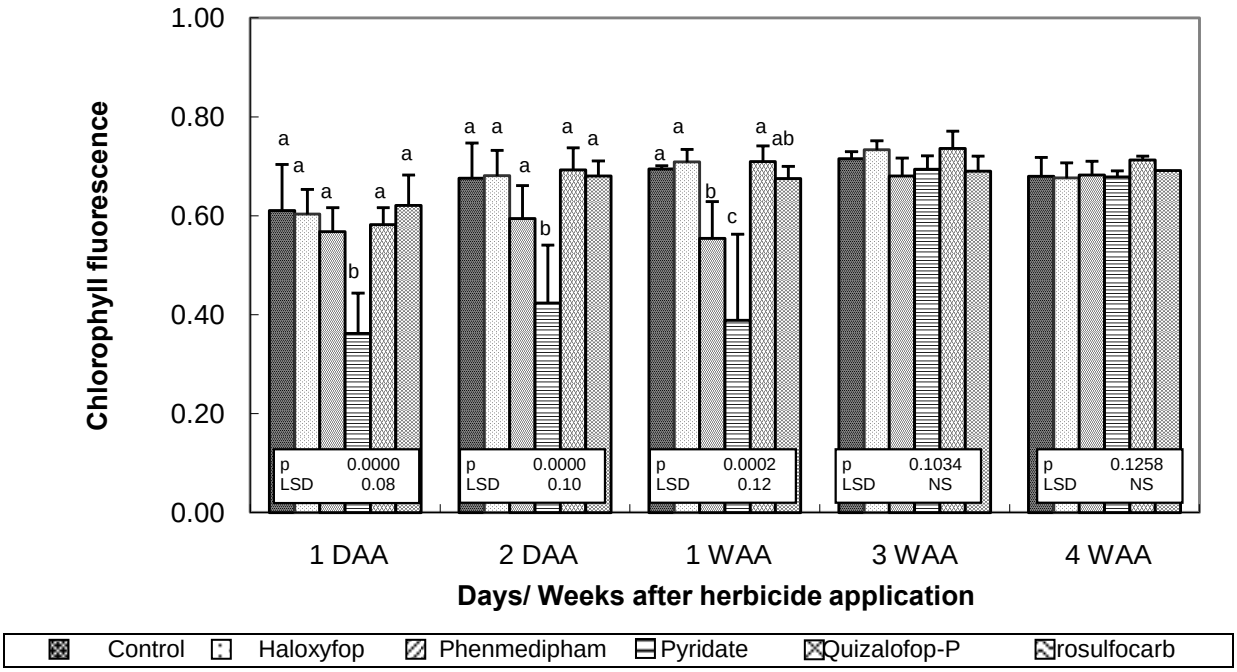


Figure 34: Effect of herbicides on chlorophyll fluorescence of artichoke leaves under dark adapted conditions in Giessen during 2nd growth phase 2006 (T = ±SD)

WAA. During 1 and 2 DAA minimum ETR was observed in case of the application of Pyridate followed by that of Phenmedipham, which were statistically different with one another and also with all other treatments under study. ETR recorded at 1 WAA also showed same response to the applied herbicides with the exception that ETR recorded by the application of Haloxyfop and Quizalofop-P were statistically at par with these values. Data recorded at 3 and 4 WAA of herbicides showed non significant response to the herbicides, where slightly different ETR values were observed but these were statistically similar with each other.

3.2.1.4 Toxicity Measurements

All the herbicidal treatments used during the first growth phase of artichoke had no significant effect at 1 DAA (see figure 36). At 1 WAA Pyridate showed an adverse affect on the artichoke leaves visible in the form of yellow leaves, chlorosis, leaf

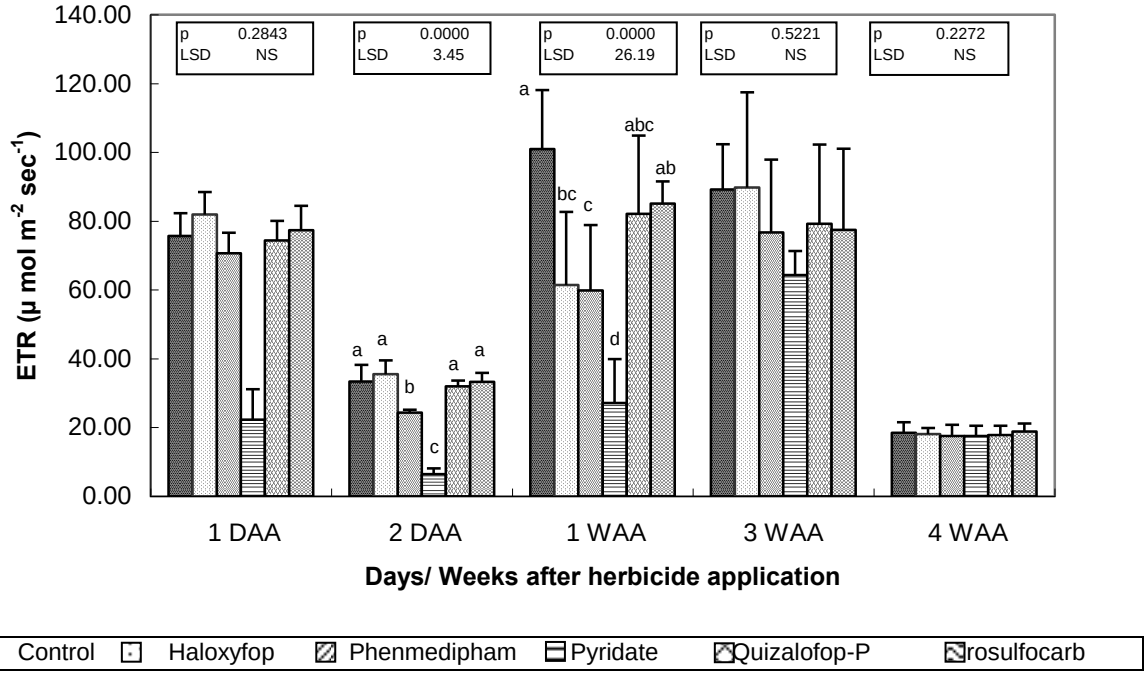


Figure 35: Effect of herbicides on electron transport rate (µ mol m^{-2} sec^{-1}) of artichoke leaves under light adapted conditions in Giessen during 2nd growth phase 2006 (T = ±SD)

burning and necrosis followed by that of Phenmedipham. It went on increasing with the same trend till 2 WAA and then started decreasing with the same trend, where Pyridate had worst effect on artichoke leaves till 4 WAA when the data were recorded for last time.

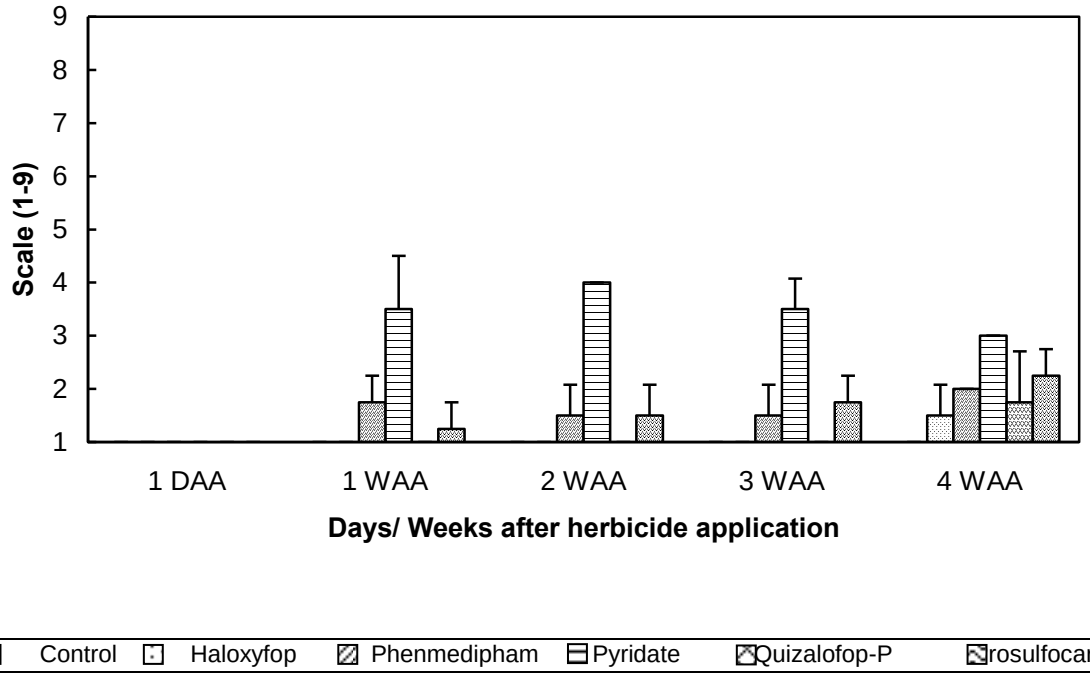


Figure 36: Effect of herbicides on toxicity measurements (scale 1-9) of artichoke in Giessen, 1st growth phase 2006 (T = ±SD)

Toxic effect of the herbicides during second phase of artichoke growth in Giessen, 2006 in figure 37 shows the same trend of toxicity as in first growth phase. Contrary to the first growth phase, this adverse affect was of a low intensity when compared with that of first growth phase. Artichoke crop showed a quick recovery against this stress of Pyridate and regained normal growth at 4 WAA (figure 37).

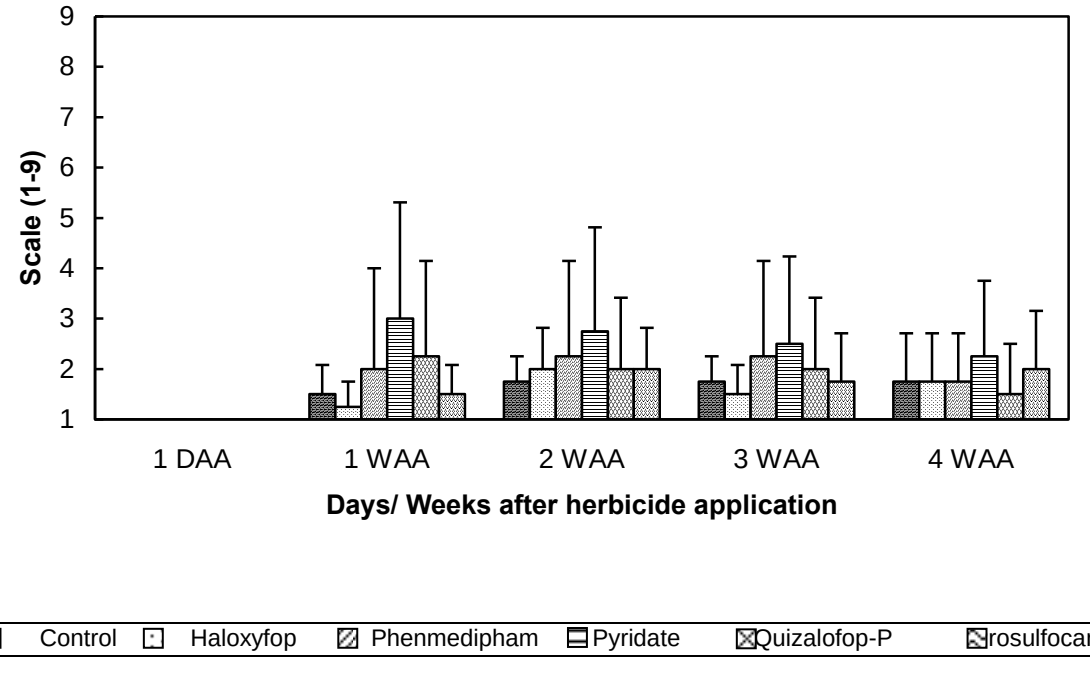
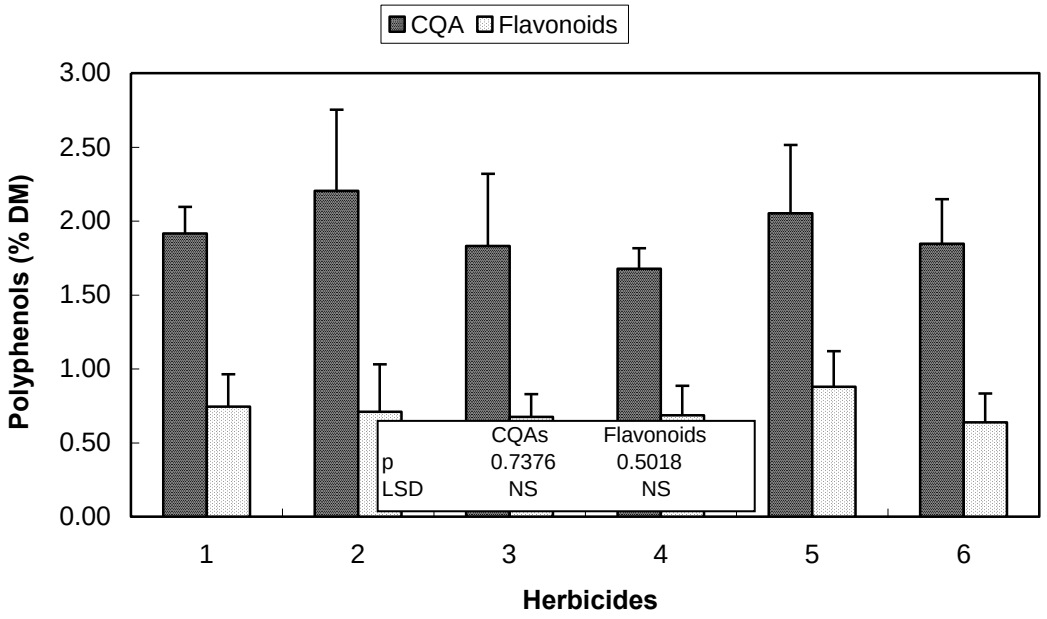


Figure 37: Effect of herbicides on toxicity measurements (scale 1-9) of artichoke in Giessen, 2nd growth phase 2006 (T = ±SD)

3.2.1.5 Caffeoylquinic acids and flavonoids

In the first year of experiments the artichoke leaves were characterized by a level of CQA of around 2 % DM and flavonoids of around 0.7 % DM (fig. 38). The figure explains that maximum caffeoylquinic acids were obtained by application of Haloxyfop followed by that of Quizalofop-P. Minimum caffeoylquinic acids were observed against the application of Pyridate followed by that of Phenmedipham. Both these were statistically at par with each other and also with that of control and all other herbicides used in the study. In the same way caffeoylquinic acids obtained by the application of Haloxyfop, Quizalofop-P, control and Prosulfocarb were statistically non significant with each other. Different herbicides used during the course of the study did not affect the flavonoids significantly, although different contents of flavonoids were observed against different treatments (figure 38).



1: Control	**2:** Haloxyfop	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P	**6:** Prosulfocarb

Figure 38: Effect of herbicides on polyphenols (% DM) of artichoke in Giessen during 1st growth phase 2006 (T = ±SD)

Concentration of caffeoylquinic acids and flavonoids in artichoke leaves in response to the herbicidal treatments during the second growth phase in Giessen 2006 tabulated in table 21 reveals that control produced minimum polyphenols (both CQA and flavonoids) that were statistically lower and different with that of all other treatments, where herbicides were applied. Minimum flavonoids were produced by the application of Phenmedipham, which were statistically same with that of all the five herbicides used in the study. Maximum CQA were obtained in case of application of Quizalofop-P that was statistically similar with that of Haloxyfop, Pyridate and Prosulfocarb (table 21)

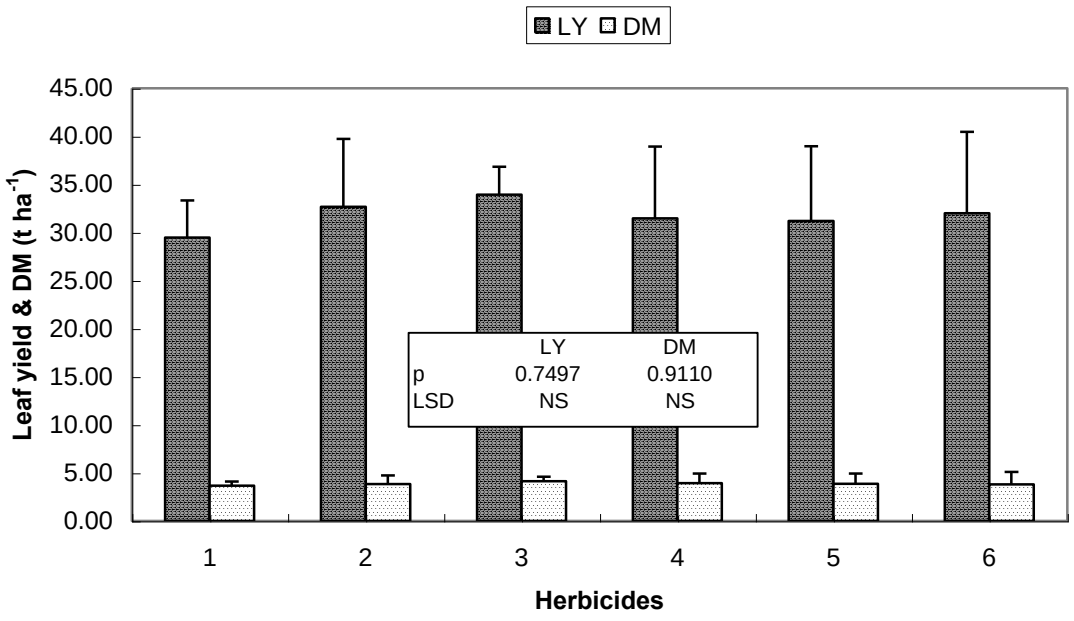
Table 21: Effect of herbicides on polyphenols (% DM) of artichoke in Giessen, 2nd growth phase 2006

Herbicidal Treatments	Caffeoylquinic Acids	Flavonoids
Control	3.27 **c**	1.19 **b**
Haloxyfop	5.15 **ab**	1.54 **a**
Phenmedipham	4.43 **b**	1.65 **a**
Pyridate	5.05 **ab**	1.59 **a**
Quizalofop-P	5.51 **a**	1.48 **a**
Prosulfocarb	5.09 **ab**	1.49 **a**
p	**0.0005**	**0.0012**
LSD	***0.91***	***0.18***

3.2.2 Field experiment Giessen 2007

3.2.2.1 Leaf yield

Leaf yield (fresh and dry matter yield in t ha^{-1}) in relation to the applied herbicides arranged in figure 39 explains that maximum yield was obtained by the application of Phenmedipham and minimum one was obtained where no herbicide was used, and that there were no significant differences among the treatments used in the study. Dry matter yield too was not affected significantly by the application of herbicides during first growth phase, 2007 in Giessen (figure 39).



1: Control **2:** Haloxyfop **3:** Phenmedipham **4:** Pyridate **5:** Quizalofop-P **6:** Prosulfocarb

Figure 39: Effect of herbicides on leaf yield (FM and DM t ha^{-1}) of artichoke in Giessen, 1st growth phase 2007 (T = ±SD)

Dry matter percentage in response to the herbicides applied to artichoke during first growth phase, 2007 in Giessen arranged in appendix A44 depicts that even though

there were slight differences among the treatments; herbicides used in the course of the study did not affect percent dry matter significantly. Appendix A45 covers the effect of the herbicidal treatments on dry matter percentage of artichoke during second growth phase, 2007. Figure in the mentioned appendix states that maximum dry matter percentage was obtained in case of the application of Pyridate that was almost same with that of application of Quizalofop-P, Prosulfocarb and control. These were slightly different and higher than that of application of Phenmedipham (minimum percent dry matter) and Haloxyfop, which showed visible similarity with each other.

Influence of the applied herbicides on leaf yield (FM and DM t ha^{-1}) is arranged in the form of table 22, which elaborates that herbicides applied to the artichoke affected leaf yield significantly on one hand and on the other hand these had no significant effect on dry matter yield. Table 22 explains that maximum leaf yield was obtained in the treatment where no herbicides were used, which was statistically similar with that of all the herbicide treatments with the exception of the application of Pyridate, where leaf yield was found to be minimum and statistically lower than that of all other treatments. As clear from the figure, different herbicides had no significant effect on the dry matter yield of artichoke, where slight differences among different treatments were observed.

Table 22: Effect of herbicides on leaf yield (FM and DM t ha^{-1}) of artichoke in Giessen, 2nd growth phase 2007

Herbicidal Treatments	Leaf Yield	
	Fresh Matter (t ha^{-1})	Dry Matter (t ha^{-1})
Control	21.50 **a**	2.45
Haloxyfop	21.39 **a**	2.36
Phenmedipham	19.43 **a**	2.06
Pyridate	14.88 **b**	1.91
Quizalofop-P	18.24 **a**	2.10
Prosulfocarb	17.49 **a**	1.99
p	**0.0500**	**0.2985**
LSD	***4.53***	***NS***

3.2.2.2 Plant stand parameters

In Giessen 2007 high level of plant height i.e. around 100 cm (first harvest) and 75 cm (second harvest) was observed (tab. 23). The herbicide application had no effect

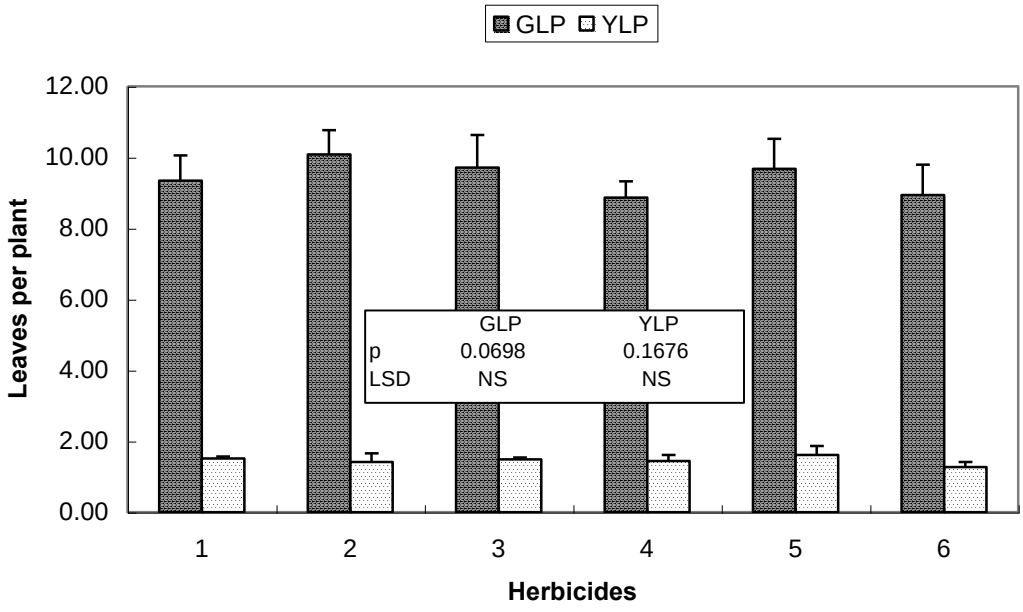
Table 23: Effect of herbicides on plant height (cm) of artichoke in Giessen, 2007

Herbicidal Treatments	1st Growth Phase	2nd Growth Phase
Control	97.9	79.1
Haloxyfop	107.2	75.9
Phenmedipham	105.9	74.4
Pyridate	102.4	72.1
Quizalofop-P	103.4	76.6
Prosulfocarb	104.6	74.4
p	**0.6868**	**0.3435**
LSD	***NS***	***NS***

on plant length at both 1st and 2nd harvest time. During first growth phase, maximum plant height was obtained in case of the application of Haloxyfop and minimum in case of control. During second growth phase maximum plant height was obtained in the treatment where no herbicides were applied. In contrast to it the application of Pyridate produced minimum plant height in artichoke.

Data relating the response of plant population per unit area to the applied herbicides during first growth phase, 2007 in Giessen presented graphically in appendix A46 explains that applied herbicides did not affect per unit population of artichoke plants significantly, although there were small differences in plant population among different treatments. Plant population per unit area in response to the applied herbicides during second growth phase in Giessen, 2007 graphically arranged in appendix A47 shows that herbicides used during the course of the study had no significant effect on the plant population of artichoke.

Maximum plant cover (%) was observed in case of control treatment, which was statistically higher and different than that of all other treatments used during the first growth phase 2007 (appendix A48). All other treatments showed minor differences in the plant cover but these were statistically same with each other and lower than that of control. Appendix A49 reflects the graphical presentation of the effect of herbicides on the plant cover of artichoke during the second growth phase in Giessen, 2007, which shows that maximum plant cover was obtained in case of control that was statistically different than that of all other treatments used. Application of Pyridate affected the leaf canopy and hence lowest plant cover was observed in this treatment, which was statistically at par with that of Phenmedipham, Quizalofop-P and Prosulfocarb.



1: Control	**2:** Haloxyfop	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P	**6:** Prosulfocarb

Figure 40: Effect of herbicides on leaves per plant of artichoke in Giessen, 1st growth phase 2007 (T = ±SD)

Effect of herbicides on number of leaves (green and yellow) per plant during the first growth phase in Giessen during 2007 arranged in figure 40 depicts that both the

parameters showed a statistically non significant response to the application of herbicides, although there were visible differences among the values of these parameters in response to the application of different herbicides. The figure shows that maximum green leaves per plant were obtained in case of application of Haloxyfop and minimum ones were observed by the application of Pyridate, which were a bit lower than that of Prosulfocarb. The figure explains that Quizalofop-P caused the maximum number of yellow leaves of artichoke in contrast with that of Prosulfocarb, which produced minimum number of yellow leaves per plant.

Maximum number of green leaves per plant was observed where Quizalofop-P was applied as post emergence herbicide and minimum green leaves per plant were produced by application of Pyridate (tab. 24). Green leaves produced by the application of Haloxyfop, Phenmedipham and control treatments were also found significantly same with that of the maximum value. Green leaves produced by the application of Prosulfocarb showed a middle value and were statistically different with that of both maximum and minimum green leaf values. Maximum number of yellow leaves per plant was obtained in case of the application of Prosulfocarb showing a less adverse effect of this herbicide, to the artichoke leaves and these were found to be statistically different with that of all other treatments used in the study. All other treatments showed slightly different but significantly same yellow leaves per plant.

Table 24: Effect of herbicides on leaves per plant of artichoke in Giessen, 2nd growth phase 2007

Herbicidal Treatments	Green Leaves per Plant	Yellow Leaves per Plant
Control	7.69 **a**	0.15 **b**
Haloxyfop	7.64 **a**	0.23 **b**
Phenmedipham	8.05 **a**	0.05 **b**
Pyridate	5.75 **c**	0.28 **b**
Quizalofop-P	7.40 **a**	0.36 **b**
Prosulfocarb	7.18 **b**	0.72 **a**
p	**0.0003**	**0.0160**
LSD	***0.79***	***0.35***

3.2.2.3 Chlorophyll fluorescence

Statistically non significant effect of herbicidal treatments on chlorophyll fluorescence for all the four dates where data were recorded is arranged in figure 4. In all cases slight differences among the chlorophyll fluorescence were observed. The data recorded at 3 and 4 WAA show that chlorophyll fluorescence recorded at these times were very close to each other and with that of control. These chlorophyll fluorescence values give an indication that the crop had recovered against the stress (although not very strong) imposed by the herbicides and visible by the slight differences among the chlorophyll fluorescence values obtained by the application of different herbicides at 1 and 2 WAA (figure 41).

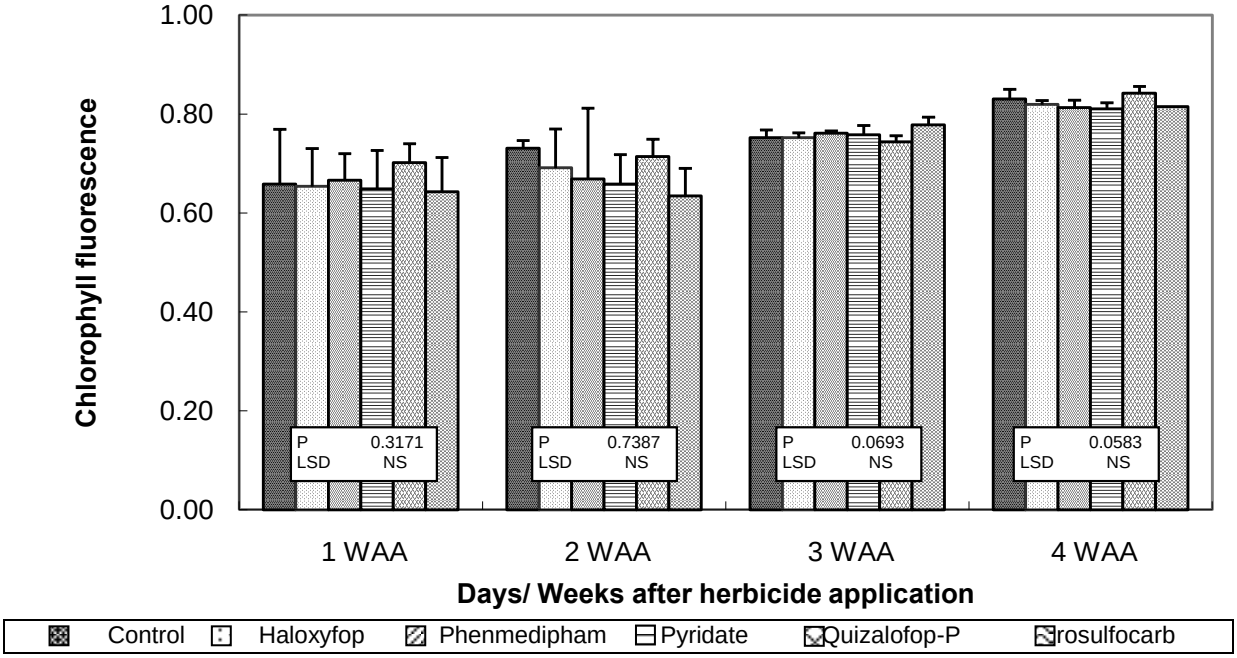


Figure 41: Effect of herbicides on chlorophyll fluorescence of artichoke leaves under light adapted conditions in Giessen, 1st growth phase 2007 (T = ±SD)

Chlorophyll fluorescence recorded under dark adapted conditions in response to the applied herbicides is arranged in the form of simple bar graph (figure 42). A close observation of the said figure shows that all the herbicidal treatments used during the first growth phase, 2007 had no significant effect on chlorophyll fluorescence under dark adapted conditions at all the dates, where data were recorded. Slight differences among the chlorophyll fluorescence values were observed only in case of

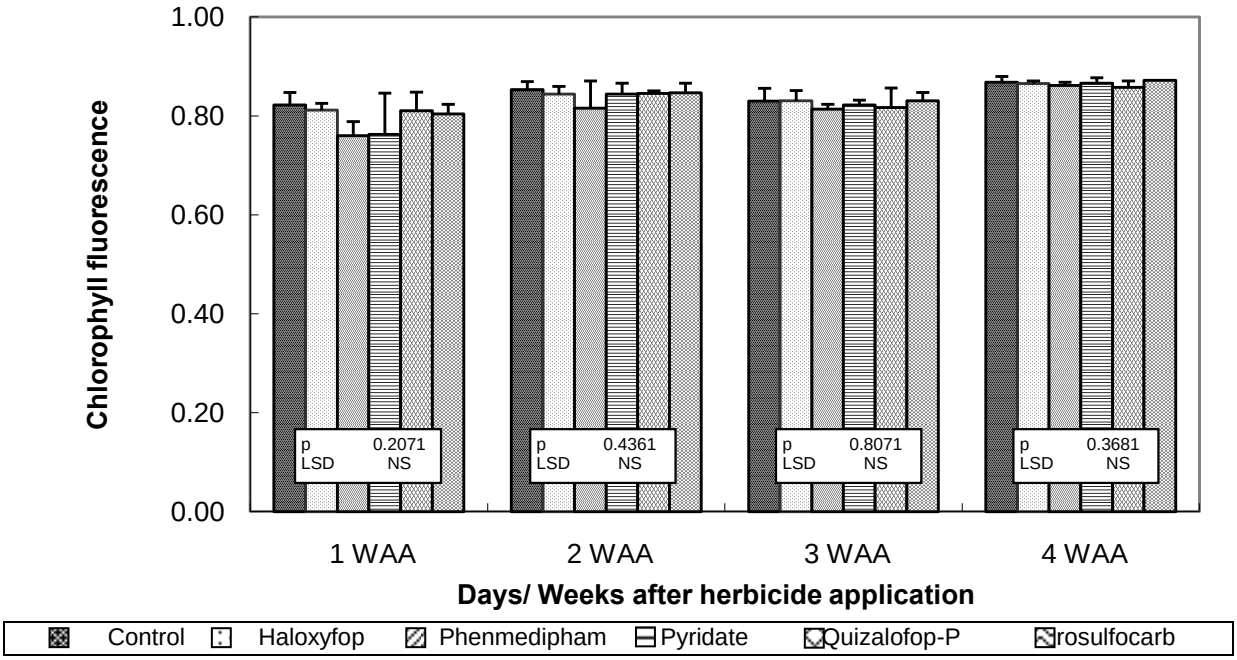


Figure 42: Effect of herbicides on chlorophyll fluorescence of artichoke leaves under dark adapted conditions in Giessen, 1st growth phase 2007 (T = ±SD)

1 WAA, where application of Phenmedipham and Pyridate showed comparatively lower chlorophyll fluorescence values but these values were statistically same.

Influence of herbicides on electron transport rate (µ mol m^{-2} sec^{-1}) during first growth phase, 2007 under direct sunlight conditions (figure 43) reflects that differences in ETR values were observed in case of application of different herbicides during first 3 weeks of application but these differences were statistically non significant. Statistically profound results were obtained in case of application of different herbicides as experimental treatments. ETR value obtained by the application of Quizalofop-P was found to be maximum that was statistically at par with that all other treatments with the exception of control and Phenmedipham, which showed significantly lower ETR values (figure 43).

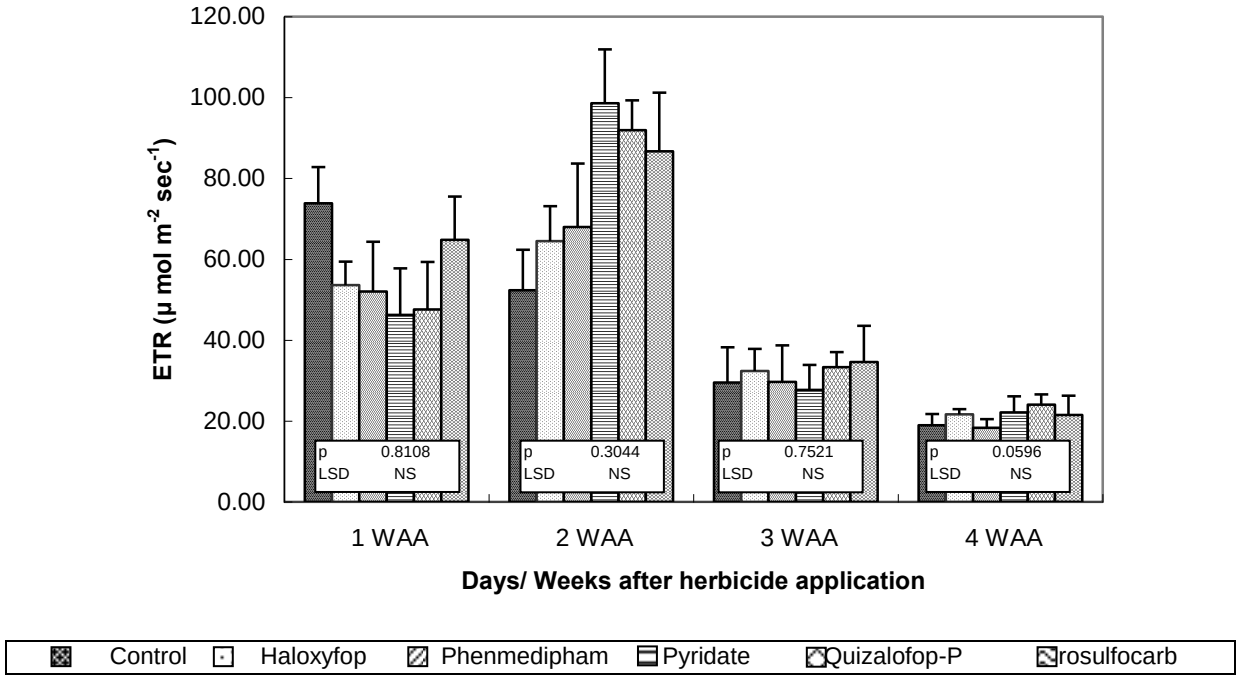


Figure 43: Effect of herbicides on electron transport rate (µ mol m^{-2} sec^{-1}) of artichoke leaves under light adapted conditions in Giessen, 1st growth phase 2007 (T = ±SD)

Pyridate affected chlorophyll fluorescence immediately after its application that is clearly visible at 1WAA, followed by a less adverse effect of Haloxyfop (figure 44). Chlorophyll fluorescence values obtained at these treatments were significantly different with each other and also with all other treatments under study including control. Adverse effect of Pyridate got worst at 2 WAA and was joined by the adverse effect of Phenmedipham that started somewhere between 1 DAA and 1WAA and was statistically at par with that of Pyridate. Both the herbicides showed statistically lower values than that of all other treatments including that of Haloxyfop, whose effect was overcame by artichoke leaves and these were statistically same with each other. Adverse effect of both Pyridate and Phenmedipham lost its intensity towards 2 WAA showing the recovery process of artichoke leaves, even though these were statistically lower than that of all other treatments under study. At 3 WAA artichoke leaves showed same trend as that of 2 WAA to the herbicides with the exception that chlorophyll fluorescence values got closer when compared with that of control, but

these were statistically lower and different with that of control and all other herbicide treatments. Chlorophyll fluorescence data recorded at 4 WAA show a complete recovery of artichoke leaves against the herbicide stress by depicting a non significant response to the treatments.

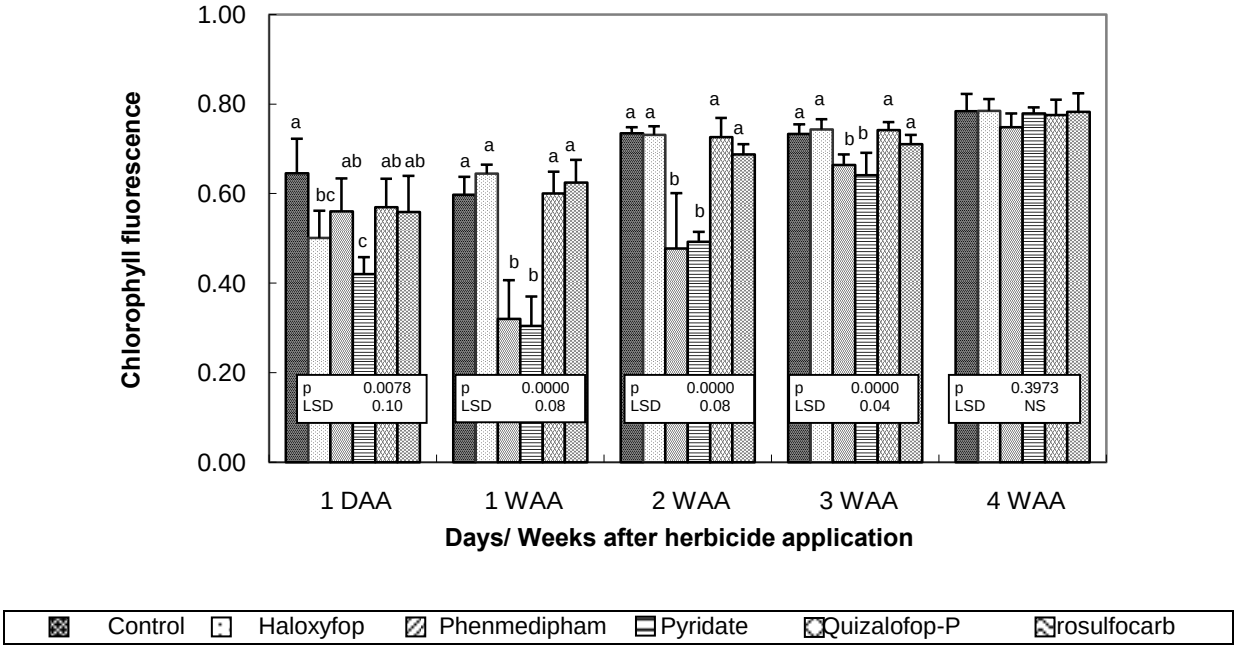


Figure 44: Effect of herbicides on chlorophyll fluorescence of artichoke leaves under light adapted conditions in Giessen, 2nd growth phase 2007 (T = ±SD)

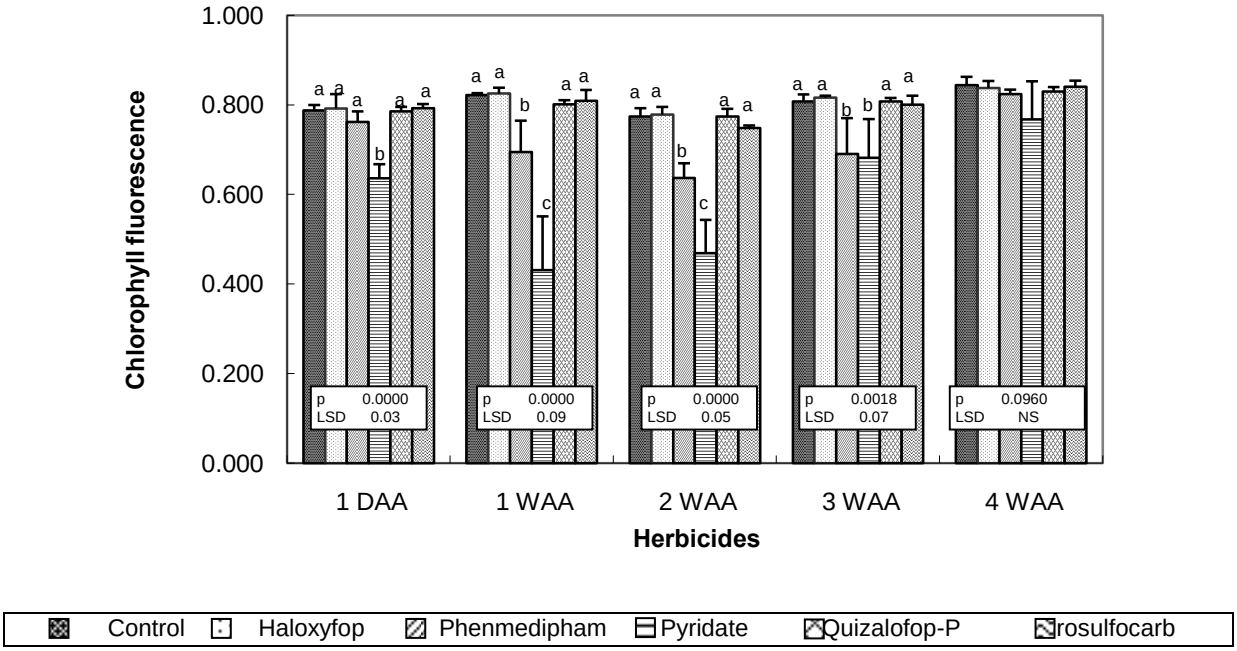


Figure 45: Effect of herbicides on chlorophyll fluorescence of artichoke leaves under dark adapted conditions in Giessen, 2nd growth phase 2007 (T = ±SD)

Herbicidal treatments affected chlorophyll fluorescence (dark adapted measurements) significantly at all dates, where data were recorded in Giessen, 2007 (see fig. 45). Figure reflects the same trend as that of light adapted conditions with the exception that under dark adapted conditions chlorophyll fluorescence at 4 WAA too showed statistically significant response to the studied treatments. Under dark adapted conditions too Pyridate and Phenmedipham affected artichoke leaves adversely right after application that got more severe till 1 WAA and then severity started to reduce till 4 WAA where Phenmedipham and Pyridate showed statistically same chlorophyll fluorescence value as that of control (figure 45).

Electron transport rate under light adapted conditions in response to the applied herbicides during second growth phase of artichoke in Giessen, 2007 is arranged in figure 46, which shows that applied herbicides did not affect the ETR at 1 DAA and 3 WAA, whereas these show a statistically significant response at 1 WAA, 2 WAA and 4 WAA. During 1 WAA recording of data Pyridate showed minimum ETR value reflecting most adverse effect of the herbicide followed by a less adverse effect of Phenmedipham and Haloxyfop, respectively which were statistically lower than that of other treatments including control. This trend continued to 2 WAA with the exception that Haloxyfop got statistically similar with that of control. At 4 WAA all the herbicides showed statistically at par ETR values giving an indication about the recovery of the crop against the stress imposed by different herbicides.

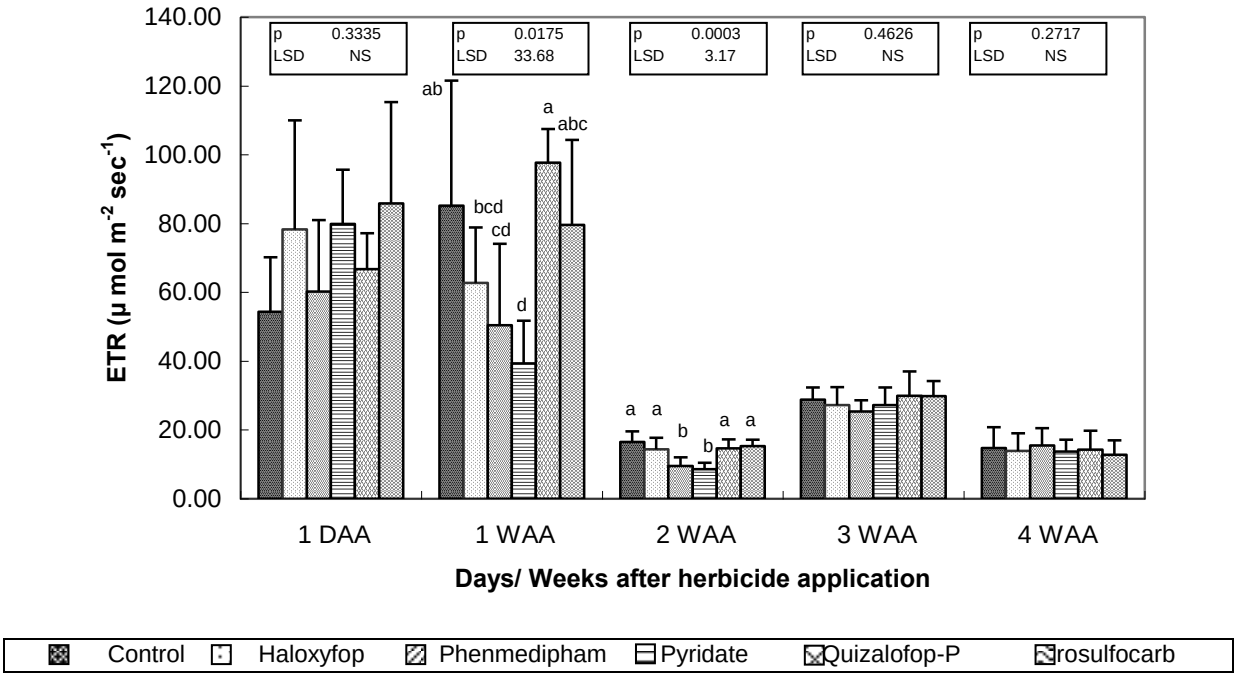


Figure 46: Effect of herbicides on electron transport rate (µ mol m^{-2} sec^{-1}) of artichoke leaves under light adapted conditions in Giessen, 2nd growth phase 2007 (T = ±SD)

3.2.2.4 Toxicity Measurements

Toxicity data collected during both growth phases of artichoke in Giessen, 2007 arranged in figures 47 and 48, respectively depicts that herbicide stress started at a period between 1 DAA and 1 WAA, where most toxic effects can be seen in plots

with application of Pyridate followed by that of Quizalofop-P and Phenmedipham. This toxic effect went on increasing till 2 WAA and then started decreasing till 4 WAA where the toxicity data were recorded for last time.

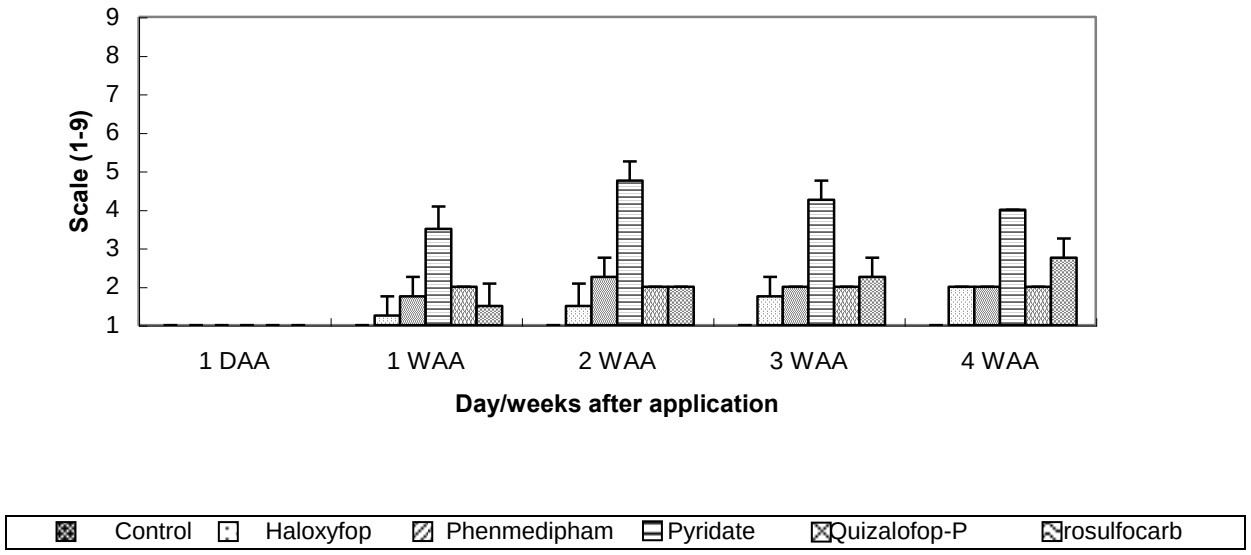


Figure 47: Effect of herbicides on toxicity measurements (scale 1-9) of artichoke in Giessen, 1st growth phase 2007 (T = ±SD)

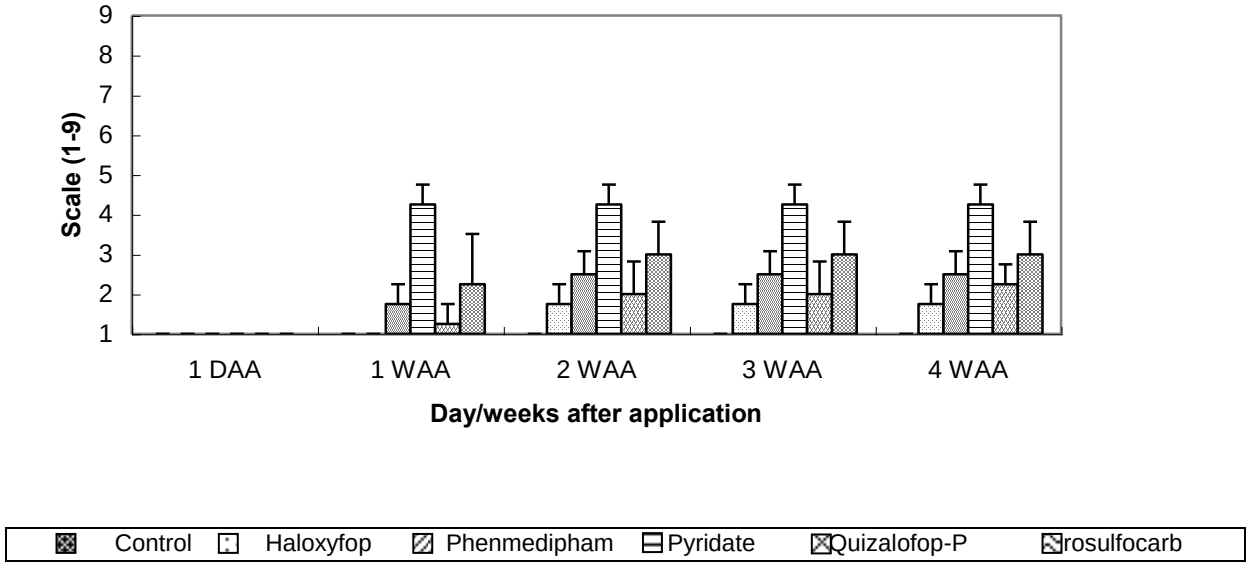


Figure 48: Effect of herbicides on toxicity measurements (scale 1-9) of artichoke in Giessen, 2nd growth phase 2007 (T = ±SD)

3.2.2.5 Caffeoylquinic acids and flavonoids

Concentrations of chlorogenic acids, caffeoylquinic acids (including chlorogenic acids), cynarosides and flavonoids (including cynarosides) in response to the herbicides used during the experimental year are presented in figure 49. Although there were minor differences in the concentration of all the compounds studied, in relation to the different herbicides used, but these were statistically at par with each other. In case of individual chlorogenic acids maximum chlorogenic acids were observed in case of application of Quizalofop-P, which were slightly higher than that of all other herbicidal treatments. Application of Haloxyfop produced minimum chlorogenic acids that were bit lower than that of all other treatments under study. Pyridate proved to be second best after Quizalofop-P for the production of chlorogenic acids and was followed by Prosulfocarb, Phenmedipham and control. Maximum caffeoylquinic acids were observed in case of application of Quizalofop-P and were found slightly higher than that of all other treatments used in the study. Minimum caffeoylquinic acids were shown by the application of Haloxyfop and were comparatively lower than that of all other treatments under study.

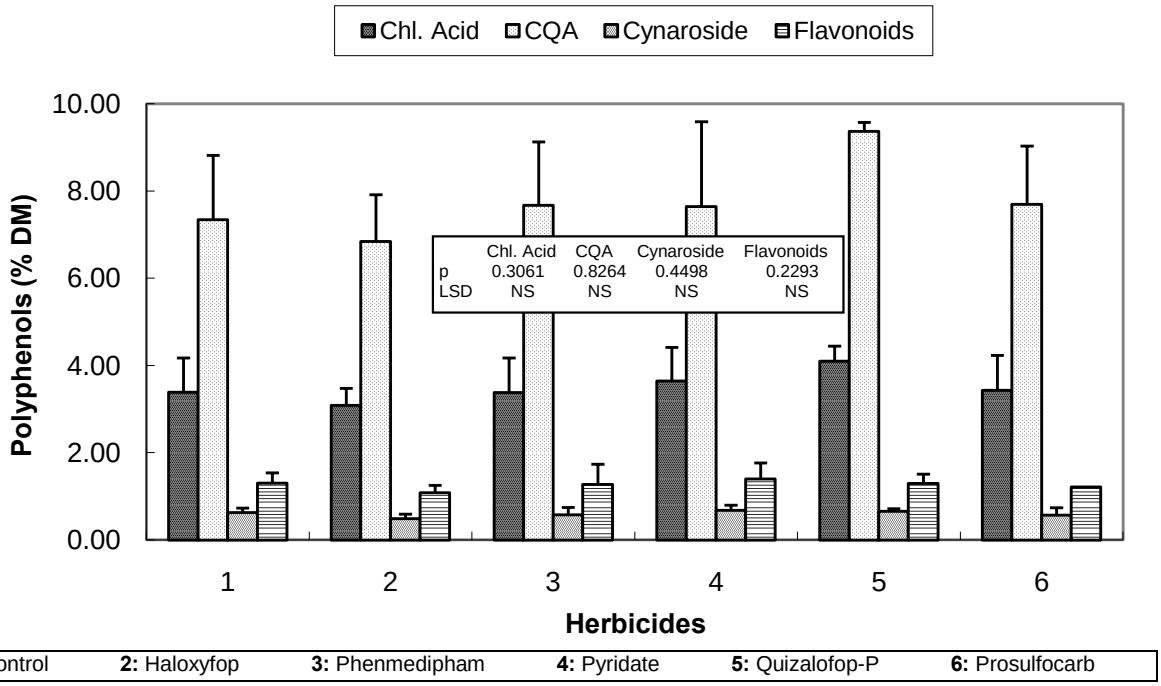


Figure 49: Effect of herbicides on polyphenols (% DM) of artichoke in Giessen, 1st growth phase 2007 (T = ±SD)

Data relating the effect of the used herbicides on the polyphenolic contents of second growth phase of artichoke leaves in Giessen, 2007 arranged in table 25 depicts that maximum chlorogenic acids were found in plots of Quizalofop-P that were statistically similar and followed by that of Phenmedipham, Pyridate, Haloxyfop and Prosulfocarb. Minimum chlorogenic acids were observed in case of control and were significantly lower than that of all the experimental treatments. Maximum caffeoylquinic acids were found against the application of Quizalofop-P, and these were statistically similar and followed by that of Phenmedipham, control, Pyridate and Haloxyfop. Minimum caffeoylquinic acids were observed in the treatment, where Prosulfocarb was applied as post emergence herbicide and was significantly same with that of Pyridate and Haloxyfop. Cynarosides and flavonoids showed a non

significant response to the experimental treatments used, and as in case of chlorogenic acids and caffeoylquinic acids maximum concentration of these compounds was observed by the application of Quizalofop-P that was lightly higher than that of Prosulfocarb and Pyridate, whereas, minimum flavonoids were produced by the application of Haloxyfop.

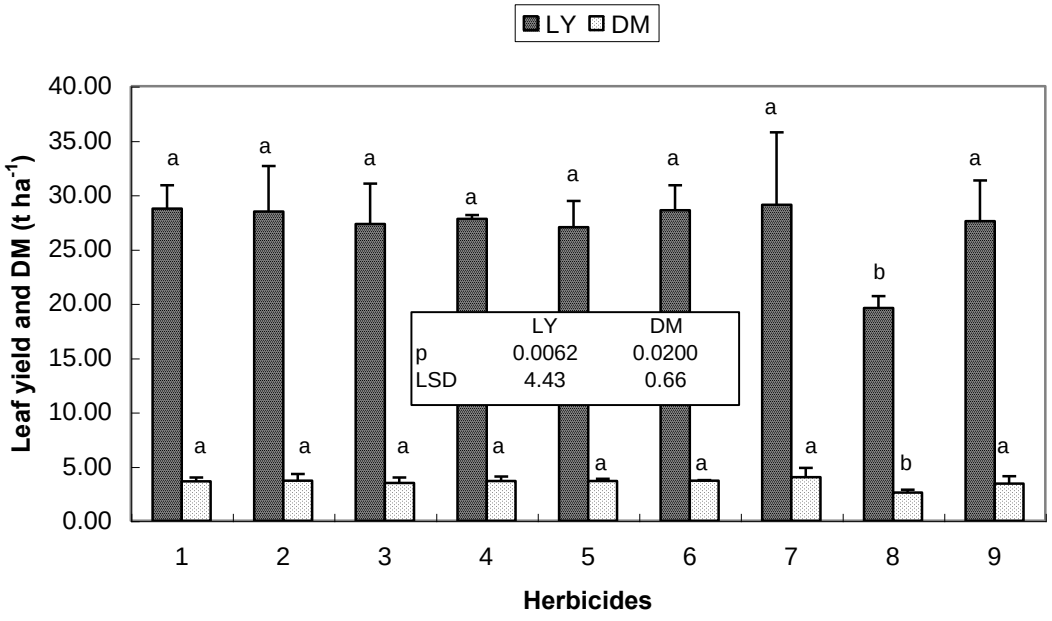
Table 25: Effect of herbicides on polyphenols (% DM) of artichoke in Giessen during 2nd growth phase 2007

Herbicidal Treatments	Chlorogenic Acids	Caffeoylquinic Acids	Cynarosides	Flavonoids
Control	2.74 **a**	3.81 **a**	0.50	1.17
Haloxyfop	2.48 **a**	3.52 **ab**	0.33	0.97
Phenmedipham	2.77 **a**	3.96 **a**	0.42	1.07
Pyridate	2.64 **a**	3.67 **ab**	0.81	1.51
Quizalofop-P	2.79 **a**	4.10 **a**	0.99	2.25
Prosulfocarb	2.19 **b**	3.05 **b**	0.53	1.71
p	**0.0127**	**0.0052**	**0.4235**	**0.3670**
LSD	***0.367***	***0.631***	***NS***	***NS***

3.2.3 Field experiment Giessen 2008

3.2.3.1 Leaf yield

A close observation of the figure 50 discloses that leaf yield (fresh and dry matter) was affected significantly by the experimental treatments. in both the cases minimum values were obtained where Aclonifen was used after the germination of artichoke plants and these were statistically different from the respective values of leaf and dry matter yields in response to the herbicidal treatments used. Maximum leaf yield



1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

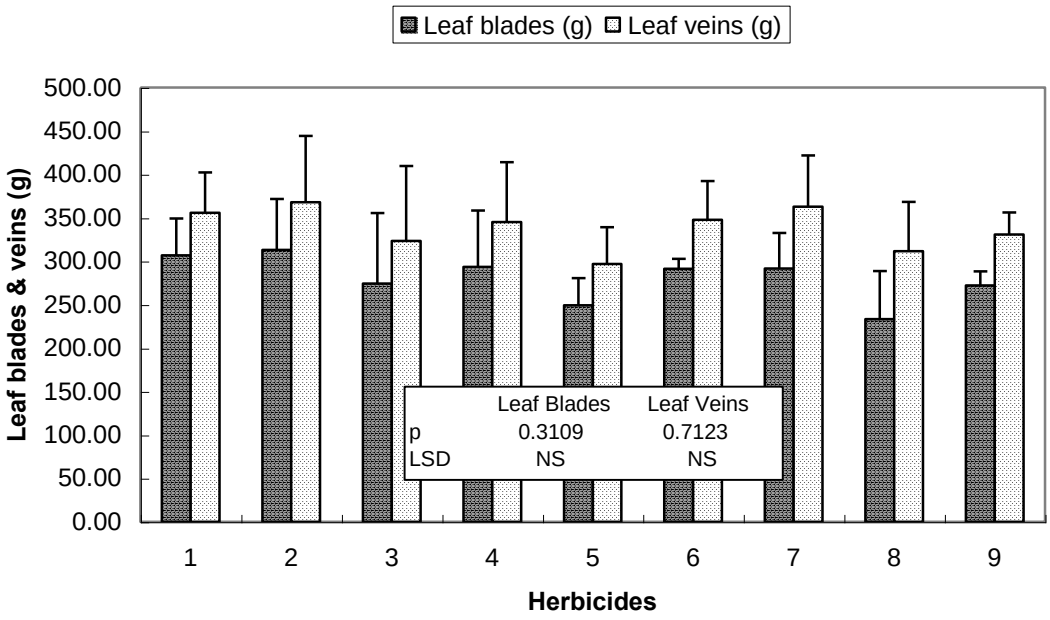
Figure 50: Effect of herbicides on leaf yield (FM and DM t ha^{-1}) of artichoke in Giessen, 1st growth phase 2008 (T = ±SD)

(29.10 t ha^{-1}) was obtained in case of application of Rimsulfuron and was found statistically at par with that of all other herbicidal treatments with the exception of Aclonifen. Leaf yield sharing the same letter ranged from 28.73 t ha^{-1} (Rimsulfuron) to 27.01 t ha^{-1} (Quizalofop-P). Dry matter yield also showed the same trend as that of leaf yield and was found maximum against the herbicide application of Rimsulfuron (4.02 t ha^{-1}) and was found significantly same with all other herbicide treatments and control with the exception of the application of Aclonifen, where minimum dry matter (2.60 t ha^{-1}) and was statistically lower than that of all other experimental treatments used during the course of the study.

Data regarding the effect of herbicides on dry matter percentage of artichoke during first growth phase in Giessen, 2008 arranged graphically in appendix A50 reflect the non significant response of dry matter percentages to the experimental treatments used in the study. Dry matter percentage of artichoke leaves as affected by herbicidal treatments during the second growth phase of artichoke in Giessen, 2008 is graphically arranged in appendix A51, which shows a significant response of the said parameter to the experimental treatments. Figure reflects that maximum percentage of dry matter was obtained in case of the application of Rimsulfuron that was statistically similar and followed by that of Quizalofop-P. Application of Clomazone produced minimum dry matter percentage that was statistically lower than that of all other experimental treatments used in the study. All other experimental treatments were statistically different from both the maximum and minimum values of dry matter and were statistically similar with one another with the exception of Quizalofop-P, which was simultaneously similar with maximum dry matter percentage value and the ones finding themselves in the middle range.

Randomly selected samples of leaves were collected and then separated into leaf veins and leaf blades and the data were collected on the basis of fresh weight (g) and percent dry matter. Leaf blades and veins (% dry matter) in relation to the applied herbicides are arranged in the form of simple bar figure in appendix A52. Figure in the said appendix states that although there were slight differences in both leaf veins and blades (% DM) these were statistically no significant among each other. Leaf blades and veins (% DM) in relation to the herbicidal treatments during the second growth phase is presented in the form of a figure tabulated in appendix A53. An overview of the figure demonstrates that leaf veins were affected significantly by the treatments used in the experiment, whereas, leaf blades showed a non significant response to these treatments. Maximum dry matter percentage in case of leaf blades was observed where Pyridate was applied, whereas minimum dry matter percentage was obtained in case of application of Clomazone. Application of Rimsulfuron produced maximum dry matter percentage in case of leaf veins that was statistically similar with that of control, Quizalofop-P and Prosulfocarb. Minimum dry matter percentage in veins of artichoke leaves was observed in case of the application of Clomazone that was statistically at par with that of Haloxyfop and Aclonifen.

Fresh weight (g) of leaf blades and veins in response to the applied herbicides during the first growth phase of artichoke is presented in figure 51. It is to see that herbicides applied to artichoke had a non significant effect on both blades and veins of artichoke leaves. Maximum weight of leaf blades were obtained in plots of Carfentrazone that was slightly higher and followed by that of control. Minimum leaf blades were produced by the application of Aclonifen and were found lower than that of the herbicidal treatments producing maximum leaf blades.



1: Control **2:** Carfentrazone **3:** Phenmedipham **4:** Pyridate **5:** Quizalofop-P
6: Prosulfocarb **7:** Rimsulfuron **8:** Aclonifen **9:** Clomazone

Figure 51: Effect of herbicides on leaf blades and veins (g) of artichoke in Giessen during 1st growth phase 2008 (T = ±SD)

Effect of herbicidal treatments on the leaf yield (FM and DM t ha^{-1}) of artichoke during the second growth phase in Giessen, 2008 is arranged in table 26. It is clear from the figure that both leaf and dry matter yield were significantly affected by the application of the herbicides during the course of the study. A close observation of the figure shows that maximum leaf yield was obtained in case of the control treatment that was significantly same and followed by that of application of Quizalofop-P, Prosulfocarb and Haloxyfop. Minimum leaf yield in this case was achieved by the application of Aclonifen that was statistically similar with that of Clomazone, Rimsulfuron and Pyridate. Leaf yield obtained by the application of Phenmedipham was in between the maximum and minimum leaf yields and was statistically same with that of minimum one. Maximum dry matter yield (t ha^{-1}) was produced in case of control treatment that was significantly similar and followed by that of Quizalofop-P,

Table 26: Effect of herbicides on leaf yield (FM and DM t ha^{-1}) of artichoke in Giessen, 2nd growth phase 2008

Herbicidal Treatments	**Leaf yield (t ha^{-1})**	**Dry Matter (t ha^{-1})**
Control	56.05 **a**	5.73 **a**
Carfentrazone	50.03 **ab**	5.21 **a**
Phenmedipham	44.73 **bc**	4.58 **ab**
Pyridate	38.41 **cd**	3.98 **bc**
Quizalofop-P	52.26 **ab**	5.54 **a**
Prosulfocarb	51.93 **ab**	5.46 **a**
Rimsulfuron	33.90 **d**	3.90 **bc**
Aclonifen	31.2 **d**	3.13 **c**
Clomazone	31.70 **d**	2.85 **c**
p	**0.0000**	**0.0000**
LSD	***9.86***	***1.13***

Prosulfocarb, Haloxyfop and Phenmedipham. Application of Clomazone reduced the dry matter to minimum level, which was statistically similar with higher dry matter values obtained by the application of Aclonifen, Rimsulfuron and Pyridate.

Data regarding the effect of the herbicide treatments on fresh yield (g) of leaf blades and veins during the second growth phase of artichoke in Giessen, 2008 in figure (52) explains that maximum leaf blades (g) were obtained by the application of Prosulfocarb that were statistically at par and followed by that of Haloxyfop, control and Phenmedipham. Rimsulfuron produced minimum leaf blades, which were statistically same with that of Aclonifen, Clomazone and Pyridate. Maximum leaf veins (g) were obtained where no herbicides were used against the weeds of artichoke, and it was statistically non significant with that of Prosulfocarb, Quizalofop-P, Haloxyfop and Phenmedipham. Other herbicides used as experimental treatments during the course of this experiment produced leaf veins (g) in the range between that of the maximum and minimum values obtained by the above mentioned herbicidal treatments.

3.2.3.2 Plant stand parameters

Non significant response of the plant height to the different herbicidal treatments used during first growth phase presented in table 27 explains that maximum height of

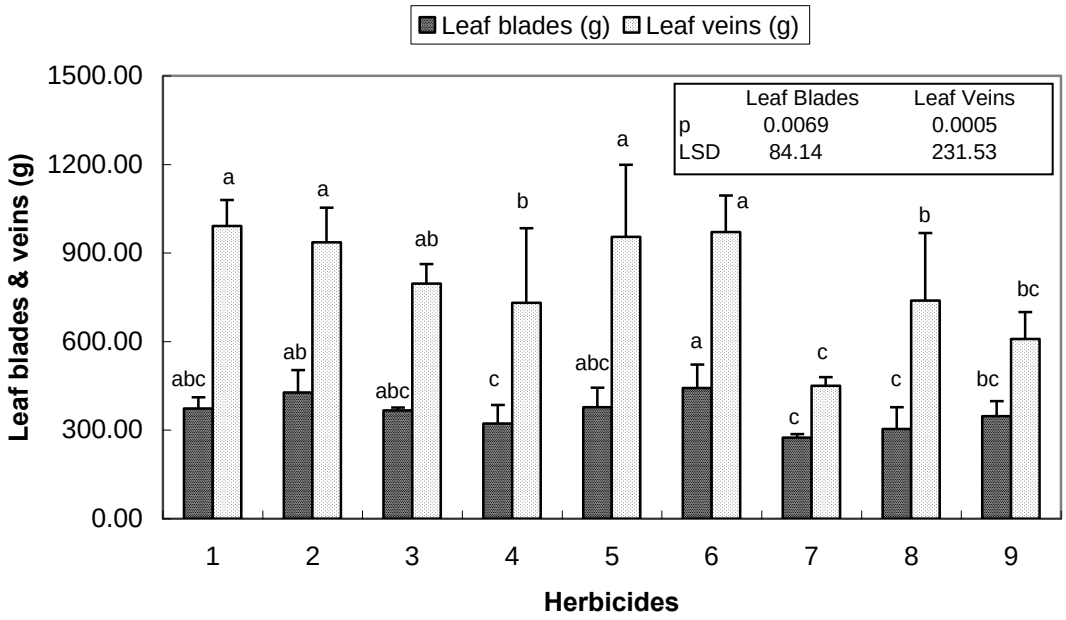


Figure 52: Effect of herbicides on leaf blades and veins (g) of artichoke in Giessen, 2nd growth phase 2008 (T = ±SD)

artichoke plants was obtained in case of application of Pyridate, whereas minimum plant height was obtained in case of application of Aclonifen. Plant heights obtained at other experimental treatments varied among both the maximum and minimum values of plant height. Statistically profound effect of the herbicides on the plant height of artichoke during second growth phase 2008 was observed (table 27). Application of Quizalofop-P produced maximum plant height that was statistically similar and followed by that of application of Prosulfocarb, Phenmedipham, and

control, Haloxyfop, Pyridate and Aclonifen. Minimum plant height was observed where Rimsulfuron was used as herbicide and was found significantly same with that of the application of Clomazone. Plant height obtained by the application of Haloxyfop, Pyridate and Aclonifen were found to be simultaneously similar with that of maximum and minimum plant heights obtained by the application of the above mentioned herbicides during the second growth phase of the artichoke in Giessen for the year 2008.

Table 27: Effect of herbicides on plant height (cm) of artichoke in Giessen, 2008

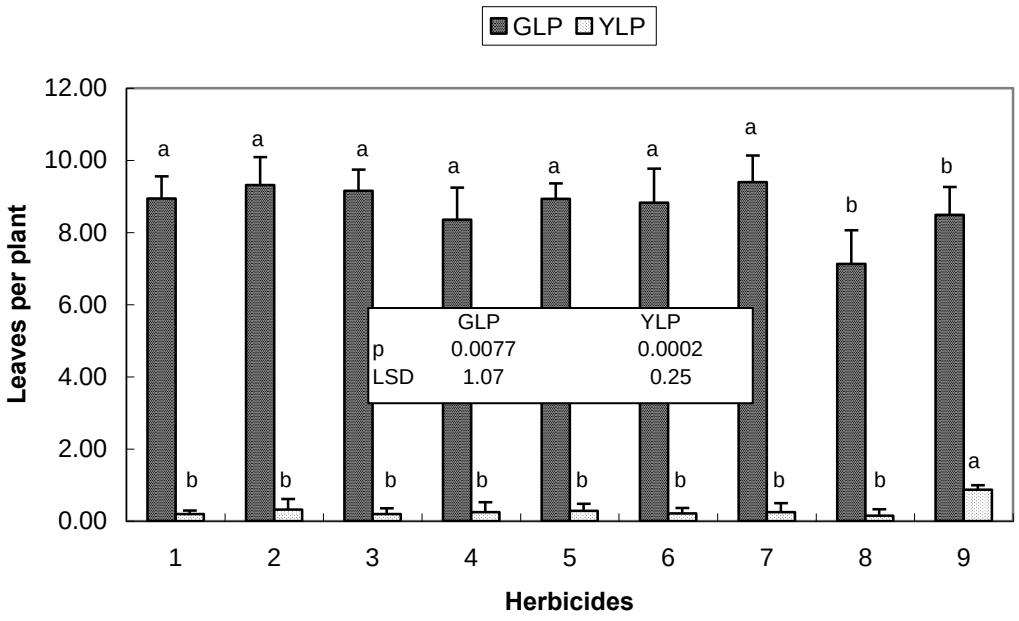
Herbicidal Treatments	**1st Growth Phase**	**2nd Growth Phase**
Control	90.47	127.93 **a**
Carfentrazone	97.80	123.45 **ab**
Phenmedipham	88.85	131.70 **a**
Pyridate	98.05	120.45 **ab**
Quizalofop-P	96.00	137.40 **a**
Prosulfocarb	94.35	128.50 **a**
Rimsulfuron	95.65	105.60 **b**
Aclonifen	88.40	117.65 **ab**
Clomazone	94.50	103.15 **b**
p	**0.3096**	**0.0342**
LSD	***NS***	***20.85***

Although slightly different heights of artichoke plant against different treatments were observed, but these were statistically similar among each other, proving that herbicides used as experimental treatments during the first growth phase of artichoke had no significant effect on the plant height of artichoke in Giessen (appendix A54). Simple bar graph in appendix A55 depicts that though different values for plant population per unit area in response to the experimental treatments were obtained, these were statistically at par with each other.

Plant cover percentage was changed statistically in the plots treated with different post emergence herbicides (appendix A56) during first growth phase of artichoke in Giessen, 2008. Maximum plant cover was observed by the application of Pyridate and minimum plant cover was seen in case of application of Aclonifen giving a hint of the adverse effect of the herbicide to artichoke leaves. Values of plant cover obtained at other experimental treatments showed slight differences among themselves but these were statistically same with each other. The plant cover values obtained by the application of these treatments were found to be statistically similar with that of the maximum and minimum values obtained and discussed in the first part of the paragraph. Contrary to the first growth maximum plant cover in second growth phase was obtained for control (where no herbicide was used) that was statistically significant and higher than that of all other treatments used in the study. It was followed by plant cover obtained by the application of Quizalofop-P that was statistically same and followed by that of Prosulfocarb and Phenmedipham. Application of Rimsulfuron produced minimum plant cover, which was significantly same with that of Clomazone and Aclonifen.

Effect of the experimental treatments on the green and yellow leaves per plant of first growth phase of artichoke in Giessen, 2008 arranged in figure 53 shows a significant effect of the applied herbicides on both the parameters presented in the figure. As

clear from the figure, maximum yellow leaves per plant were observed in case of application of Clomazone that were statistically higher and different than that of all other treatments in the study. This value of yellow leaves showed the toxic effect of the herbicide on the artichoke during the study period. Maximum number of green leaves in the studied experiment was observed by the application of Rimsulfuron, which was statistically at par with that of all other treatments under study, with the exception of Aclonifen and Clomazone. Application of Aclonifen produced minimum number of green leaves per plant and these were statistically same with that of the application of Clomazone, which showed slightly higher number of green leaves per plant of artichoke during the experimental period of 2008.



1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Figure 53: Effect of herbicides on leaves per plant of artichoke in Giessen, 1st growth phase 2008 (T = ±SD)

Leaves per plant (green and yellow) of artichoke in response to the herbicides used during second growth phase of artichoke showed statistically significant response to the experimental treatments used (table 28). Maximum green leaves per plant were observed in case of the control treatment that were statistically similar and followed by that of Prosulfocarb, Quizalofop-P, Haloxyfop and Phenmedipham. Minimum number of green leaves per plant was obtained in case of application of Clomazone giving a clue of adverse effect of the herbicide. This number of green leaves per plant showed statistical similarity with that of Rimsulfuron, Aclonifen and Pyridate.

Table 28: Effect of herbicides on leaves per plant of artichoke in Giessen, 2nd growth phase 2008

Herbicidal Treatments	Green Leaves per Plant	Yellow Leaves per Plant
Control	9.91 **a**	3.28 **b**
Carfentrazone	9.37 **abcd**	2.87 **b**
Phenmedipham	8.82 **abcd**	3.06 **b**
Pyridate	7.11 **de**	4.36 **b**
Quizalofop-P	9.66 **abc**	2.85 **b**
Prosulfocarb	9.78 **ab**	3.02 **b**
Rimsulfuron	7.45 **bcde**	3.12 **b**
Aclonifen	7.32 **cde**	4.21 **b**
Clomazone	5.80 **e**	6.64 **a**
p	**0.0121**	**0.0012**
LSD	***2.39***	***1.63***

3.2.3.3 Chlorophyll fluorescence

Chlorophyll fluorescence (µ mol CO_2 m^{-2} sec^{-1}) in response to the herbicides used as experimental treatments during first growth phase of artichoke, under light adapted conditions of Giessen presented in figure 54 depicts that chlorophyll fluorescence was significantly affected by the herbicides during all the dates where data were recorded. A close observation of the figure shows that Pyridate produced an adverse effect on the leaves of artichoke just after its application, as visible by the chlorophyll fluorescence value obtained at 1 DAA, which is statistically lower than that of all other

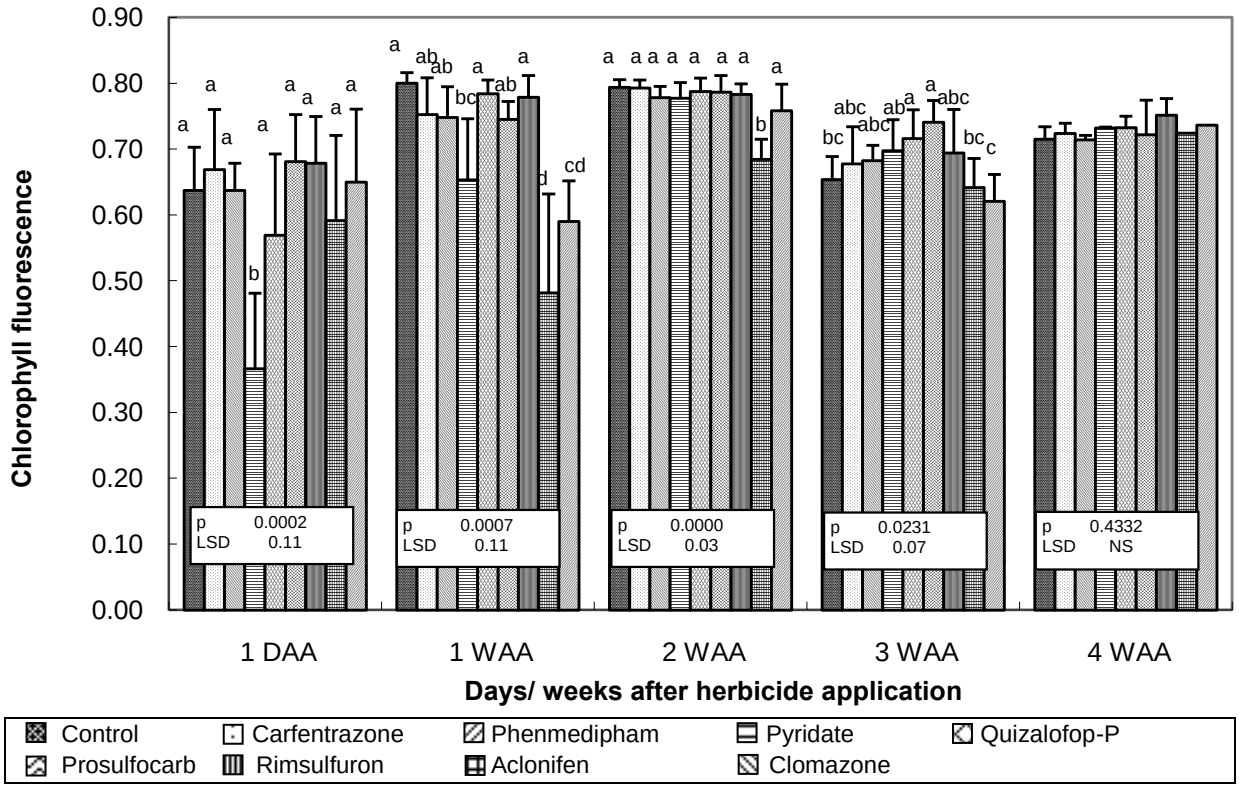


Figure 54: Effect of herbicides on chlorophyll fluorescence of artichoke leaves under light adapted conditions in Giessen, 1st growth phase 2008 (T = ±SD)

treatments. All other experimental treatments at this time showed a non significant response to the chlorophyll fluorescence, when compared with that of control. The data presented at 1 WAA shows 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 value obtained at 2 WAA. 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 3 WAA showed a mixed response to the applied herbicides. The data recorded at 4 WAA shows a non significant response of the chlorophyll fluorescence value to the herbicides used giving a clue about the recovery of the crop against the adverse effects of the herbicides.

Chlorophyll fluorescence in relation with the herbicidal treatments used during first growth phase of artichoke under dark adapted conditions of Giessen, 2008 elaborates the immediate adverse effect of Pyridate as visible at 1 DAA, which was statistically lower than that of all other treatments (figure 55). At 1 WAA, Aclonifen started showing its adverse effect by showing a statistically lower value of chlorophyll fluorescence. The effect went on increasing till 2 WAA, and then crop started recovery process visible through 3 WAA and completed the recovery process, visible in the chlorophyll fluorescence data recorded at 4 WAA, where no statistical difference among different herbicide treatments was observed, when compared with that of the control.

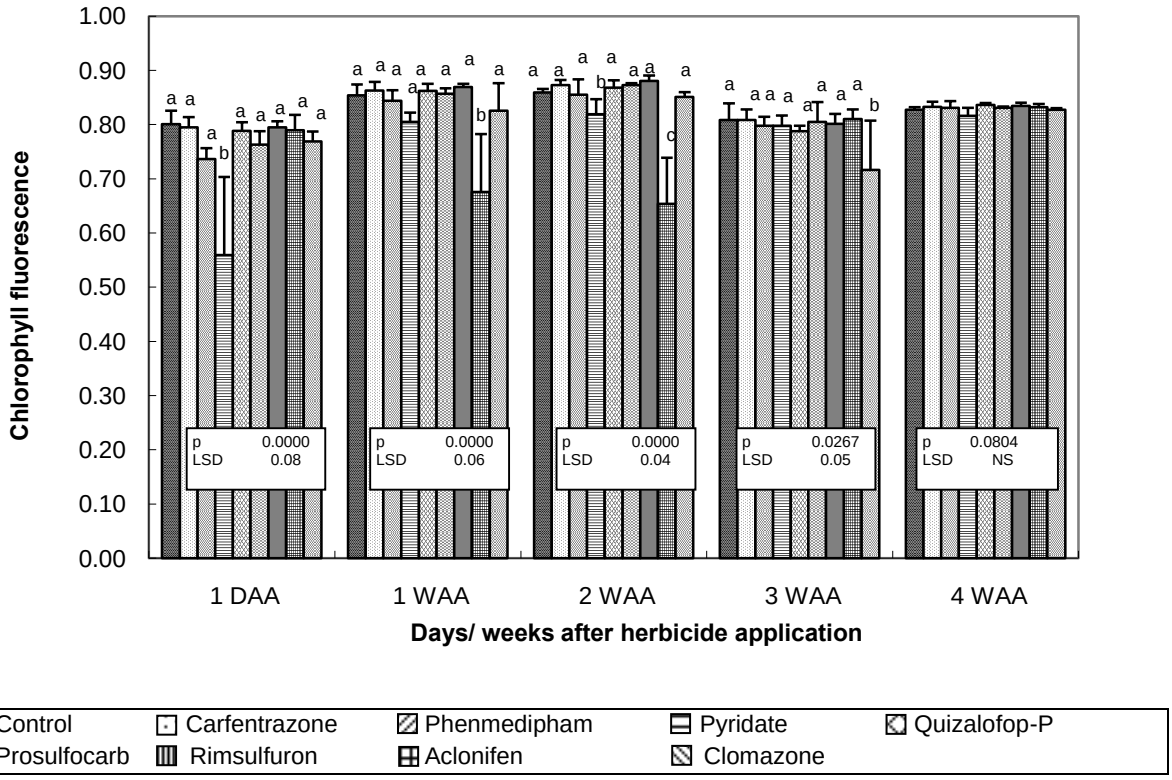


Figure 55: Effect of herbicides on chlorophyll fluorescence of artichoke leaves under dark adapted conditions in Giessen, 1st growth phase 2008 (T = ±SD)

Electron transport rate (µ mol m^{-2} sec^{-1}) affected by the herbicides used during the course of the experiment for the first growth phase of artichoke under light adapted

conditions of Giessen during 2008 is arranged in the figure 56. Figure shows a statistically significant response of ETR to the herbicidal treatments at 1 WAA and a non significant response at all other times of data recording. In spite of the non significant response, there were visible differences among the ETR at all the dates with the exception of 4 WAA, which shows slight differences among these values in response to the different herbicides used during the year. At 1 DAA Pyridate showed minimum ETR value whereas maximum ETR value was observed at the treatment of Aclonifen followed by that of Clomazone. At 1 WAA, minimum value of ETR was observed in case of application of Aclonifen that was nearly similar with that of Clomazone, whereas application of Haloxyfop showed a maximum ETR value. ETR obtained at 3 WAA and 4 WAA showed a mixed response to the applied herbicides as there was no specific trend in the response of artichoke to the applied herbicides in connection with ETR.

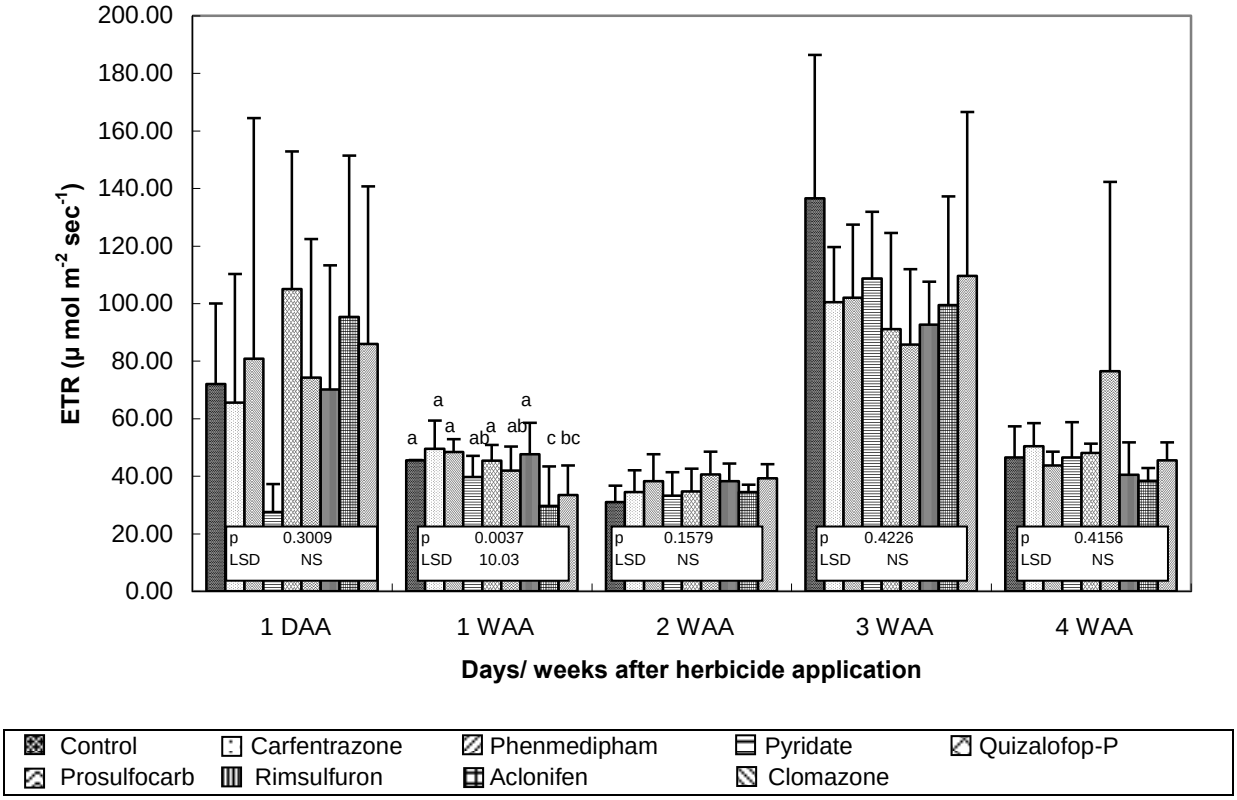


Figure 56: Effect of herbicides on electron transport rate (µ mol m^{-2} sec^{-1}) of artichoke leaves under light adapted conditions in Giessen, 1st growth phase 2008 (T = ±SD)

Maximum chlorophyll fluorescence at 1 DAA during second growth phase 2008 was obtained by the application of Rimsulfuron, which was slightly higher when compared with that of control (see figure 57). Minimum value of Chlorophyll fluorescence in this case was obtained by the application of Aclonifen that was almost similar with that of all of experimental treatments with the exception of Rimsulfuron, Clomazone, Haloxyfop and Phenmedipham. At 1 WAA chlorophyll fluorescence value obtained by the application of both Haloxyfop and Prosulfocarb was statistically similar with that of control, which at the same time was also significantly similar with that of Phenmedipham and Rimsulfuron. At 3 WAA minimum value of chlorophyll fluorescence was observed in case of application of Aclonifen with a slightly higher

one at the application of Clomazone and these two were significantly same with each other. Same was true for Pyridate and Phenmedipham, which show the same trend and show statistically lower chlorophyll fluorescence values in comparison with that of the herbicides showing most adverse effects. This trend of the response of chlorophyll fluorescence values continued with a very little variation till 4 WAA, where data were recorded for the last time but the values of the chlorophyll fluorescence started increasing for the herbicides that show an adverse effect and keep getting closer to that of the value obtained at control treatment. 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 chlorophyll fluorescence when compared with that of control at 4 WAA. At this time these two herbicides showed statistically different chlorophyll fluorescence between one another too.

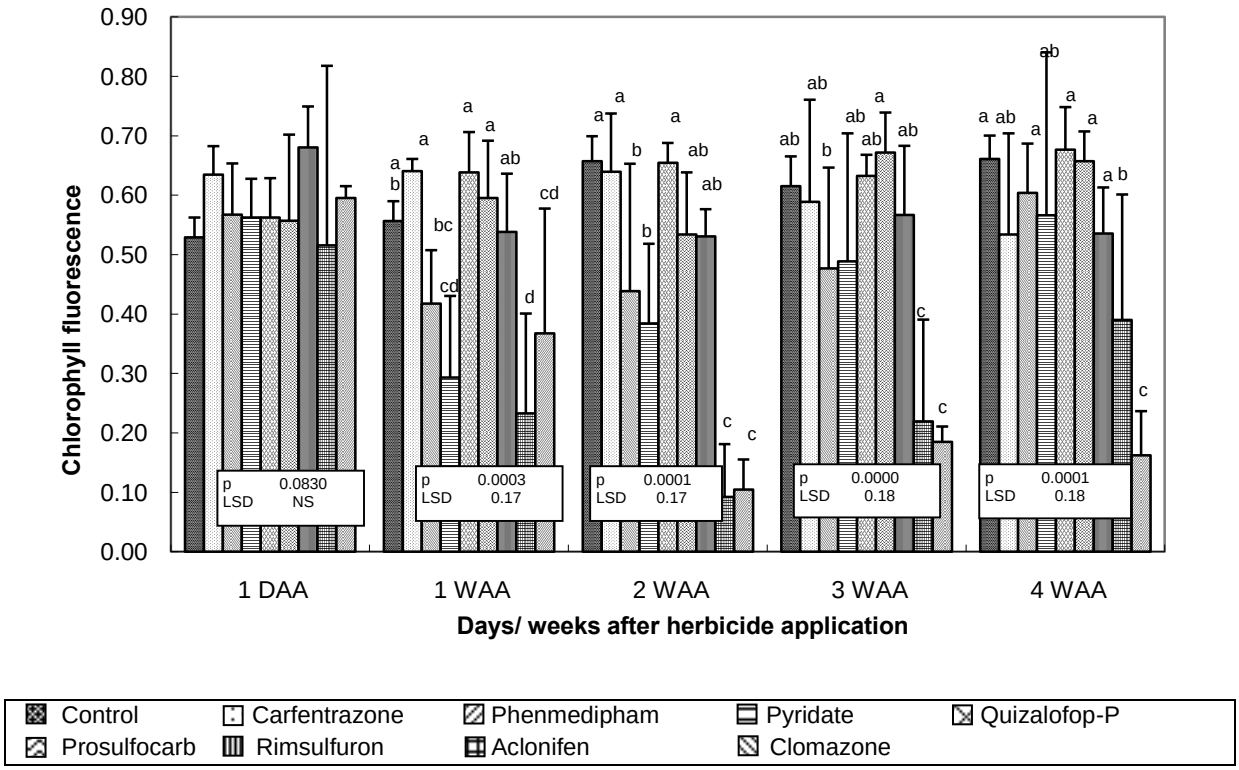


Figure 57: Effect of herbicides on chlorophyll fluorescence of artichoke leaves under light adapted conditions in Giessen, 2nd growth phase 2008 (T = ±SD)

Statistically significant effect of the herbicides on the chlorophyll fluorescence measured under dark adapted conditions in Giessen during second phase of artichoke growth in 2008 presented in figure 58 explains that Pyridate showed a negative impact on the leaves immediately after its application (1 DAA), which got worst at 1 WAA and was joined by Phenmedipham, Aclonifen and Clomazone that were statistically similar with each other and toxic to artichoke in a descending order. This trend was changed at 2 WAA when the worst adverse effects of the herbicides were observed by the application of Clomazone and reduced towards Aclonifen, Pyridate and Phenmedipham in a consecutive order. This trend maintained itself till 4 WAA where data were recorded for the last time. Chlorophyll fluorescence values for all other experimental treatments were statistically non significant when compared

with that of control, although there were minor differences among the values at all the times when data were recorded.

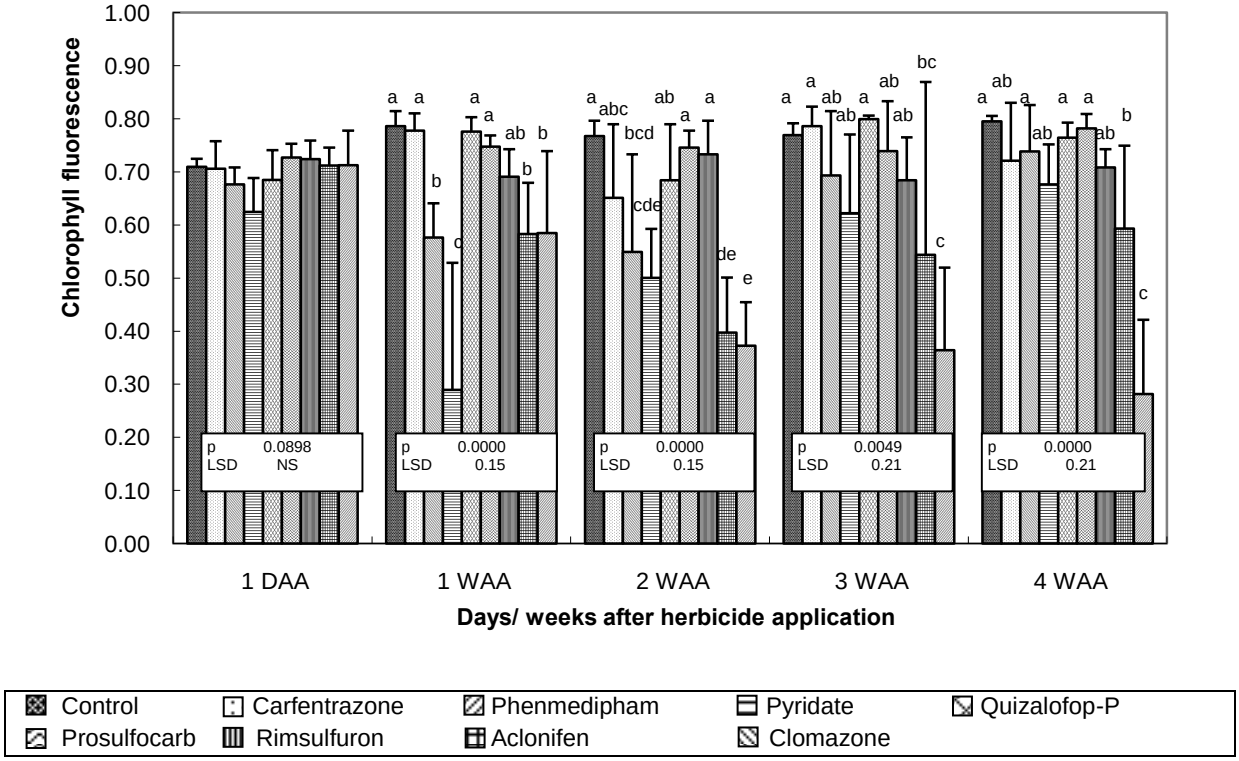


Figure 58: Effect of herbicides on chlorophyll fluorescence of artichoke leaves under dark adapted conditions in Giessen, 2nd growth phase 2008 (T = ±SD)

Herbicides used during second phase of artichoke growth had a statistically significant effect on ETR (light adapted measurements) at all the times when data were recorded with the exception of 1 DAA and 1 WAA (figure 59). Minimum ETR value at 1 WAA was obtained in case of application of Pyridate and was found nearly similar with that of all other herbicides with the exception of Aclonifen, which showed visibly higher ETR value. ETR values recorded from 2 to 4 WAA of herbicides show that Clomazone showed most adverse effect on the leaves of artichoke and it was statistically different and followed by that of Aclonifen, Pyridate and Phenmedipham. This adverse effect of Clomazone and Aclonifen lasted till 4 WAA with a slightly different trend of Pyridate and Phenmedipham, which changed their order of adversity at the later data measurements.

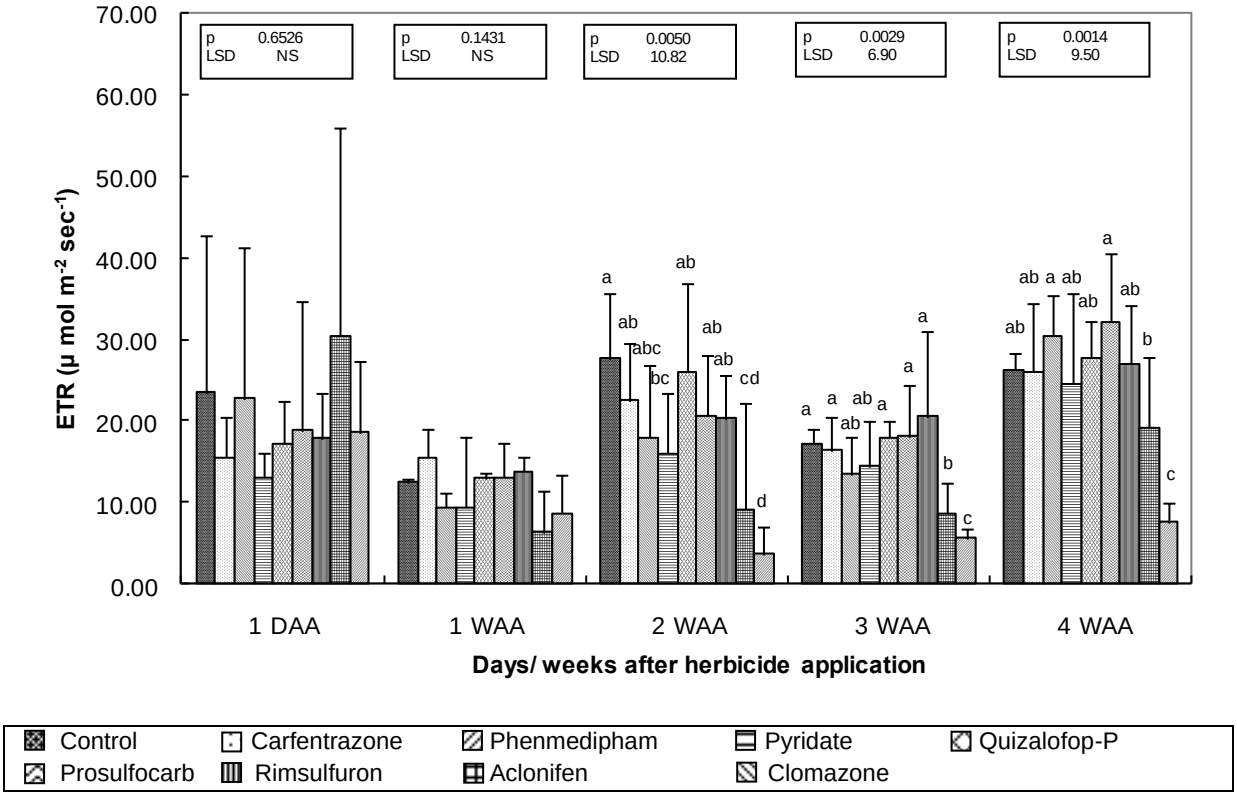


Figure 59: Effect of herbicides on electron transport rate (µ mol m^{-2} sec^{-1}) of artichoke leaves under light adapted conditions in Giessen, 2nd growth phase 2008 (T = ±SD)

3.2.3.4 Toxicity Measurements

No visual toxicity symptoms of the applied herbicides at 1 DAA (first growth phase, 2008) were observed at 1 DAA and these started to appear at 1 WAA and showed a different response depending on the types of the herbicides (figure 60). Pyridate showed yellowish leaves at 1 WAA and the intensity of the toxicity increased till 2 WAA and then it did maintain this level till 4 WAA, where data were recorded for the last time. The same trend was shown by the treatment where Clomazone was applied to the artichoke leaves. In contrast to these two herbicides the adverse effect of Aclonifen got on increasing till 3 WAA and then at 4 WAA showed the same value as that at 3 WAA. Other herbicides used during the course of the study showed a mixed response but any of these did not have any severe adversity that was visible in the form of yellowing of leaves or so on.

A more severe adverse impact of Clomazone starting at 1 WAA and increasing till 4 WAA was observed during second growth phase (see figure61). Over all toxic effect of all the herbicides during this growth phase was more severe when compared with that of the first growth phase during the year 2008. Toxic effect of Aclonifen also showed the same trend as that by Clomazone, whereas toxic effect of Pyridate increased till 3 WAA and at 4 WAA it showed a comparatively lower toxicity. Haloxyfop, Phenmedipham and Quizalofop-P also showed a little toxicity during this phase of the artichoke growth. Rimsulfuron too had a severe toxic effect on the leaves of artichoke during this phase, which showed same trend as that of Aclonifen and Clomazone (figure 61).

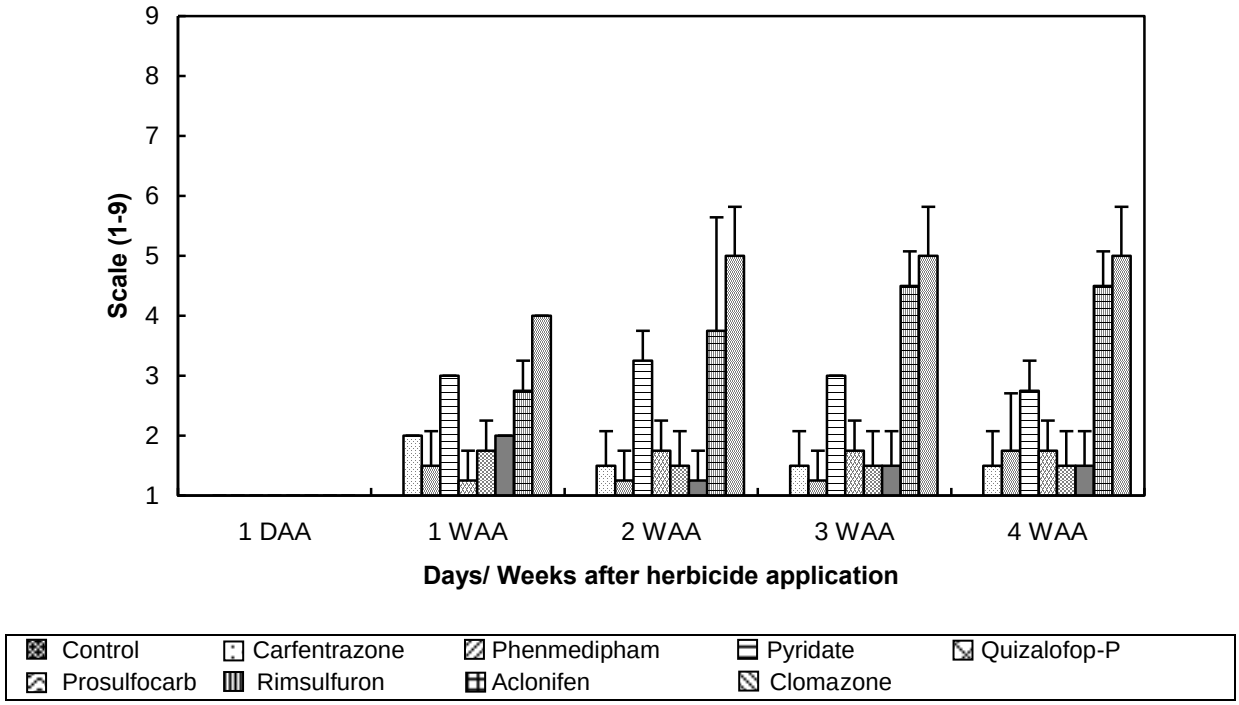


Figure 60: Effect of herbicides on toxicity measurements (scale 1-9) of artichoke in Giessen, 1st growth phase 2008 (T = ±SD)

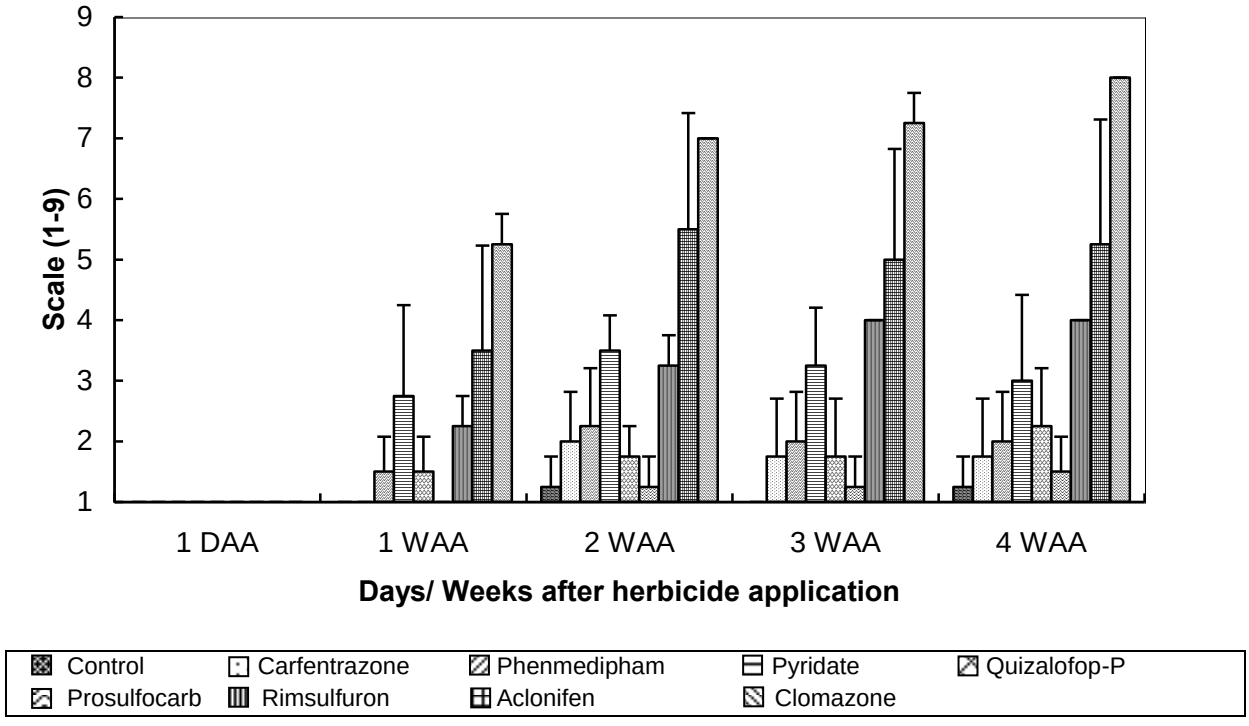
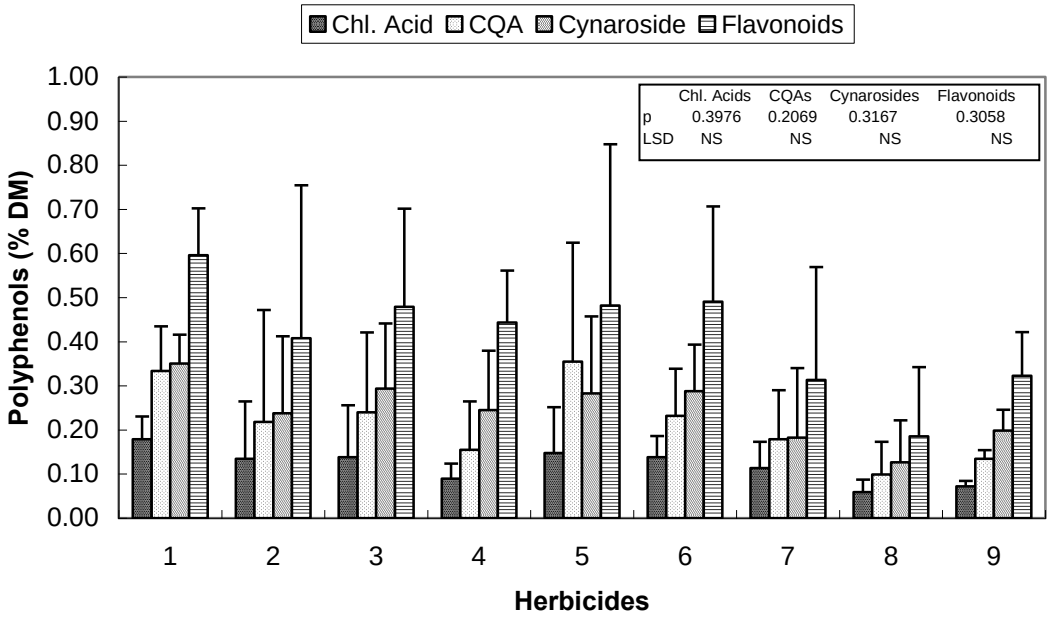


Figure 61: Effect of herbicides on toxicity measurements (scale 1-9) of artichoke in Giessen, 2nd growth phase 2008 (T = ±SD)

3.2.3.5 Caffeoylquinic acids and flavonoids

Non significant response of caffeoylquinic acids and flavonoids (% DM) to the herbicidal treatments at 1 WAA during the first growth phase of artichoke depicted in figure 62 shows that maximum chlorogenic acids were obtained by the treatment where no herbicides were used. Maximum caffeoylquinic acids were obtained in case of application of Quizalofop-P. Application of Aclonifen produced minimum percentage of both the chlorogenic acids and caffeoylquinic acids at this time of growth. Other experimental treatments used during the course of the study showed a mixed response to the applied herbicides.

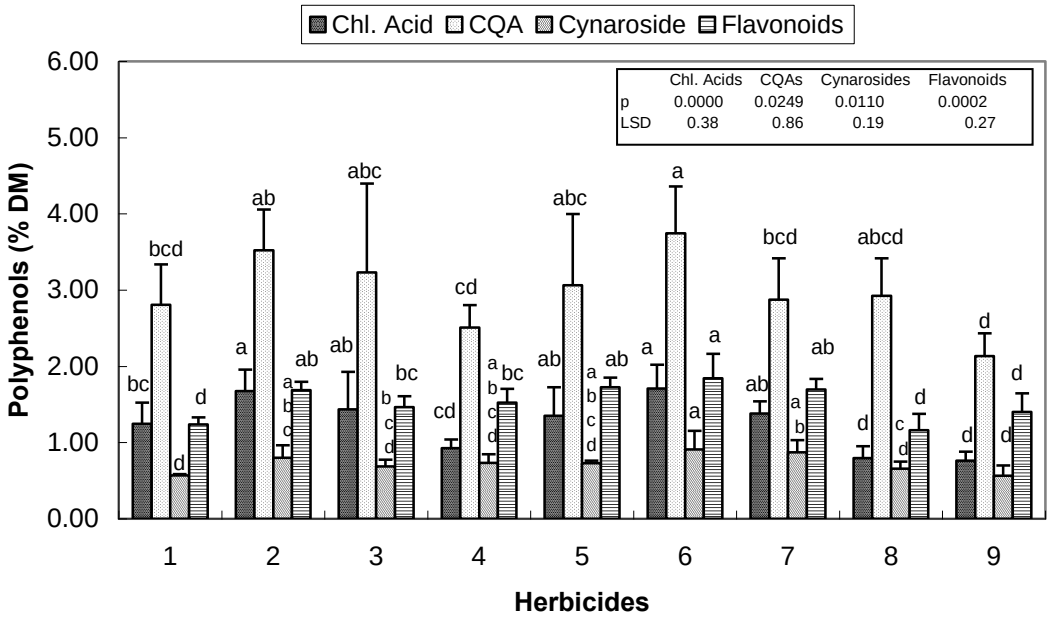


1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Figure 62: Effect of herbicides on polyphenols (% DM) of artichoke in Giessen, 1WAA 1st growth phase 2008 (T = ±SD)

Polyphenols in relation to the post emergence herbicides used at 2 WAA during first growth phase of artichoke arranged in figure 63 explain that all the polyphenols were significantly affected by the herbicides used as experimental treatments. A close observation of the figure explains that application of Prosulfocarb produced maximum concentration of all the studied polyphenols (chlorogenic acids, caffeoylquinic acids, cynarosides and flavonoids), whereas application of Clomazone produced minimum concentration of all these compounds with the exception of Flavonoids, which were found to be minimum in case of application of Aclonifen. Chlorogenic acids, caffeoylquinic acids and cynarosides produced by the application of Carfentrazone Phenmedipham and Quizalofop-P were recorded, as significantly same as that of the treatments, where maximum concentration of these compounds was produced. In contrast to that, application of Pyridate, Aclonifen and Rimsulfuron led to similar concentration of polyphenols with that of minimum concentration of these compounds. Control treatment, where no herbicides were applied produced comparatively lower amounts of all the polyphenols studied during the course of the experiment. Flavonoids showed a different response when compared with that of other polyphenols. Concentration of flavonoids by the application of Carfentrazone,

Quizalofop-P and Rimsulfuron was statistically similar with that of the higher concentration of flavonoids, and on the other hand control and Clomazone produced flavonoids significantly at par with that of the minimum ones. Other treatments used in the study showed a mixed response to the concentration of flavonoids, which at these concentrations were found between the maximum and minimum flavonoids.

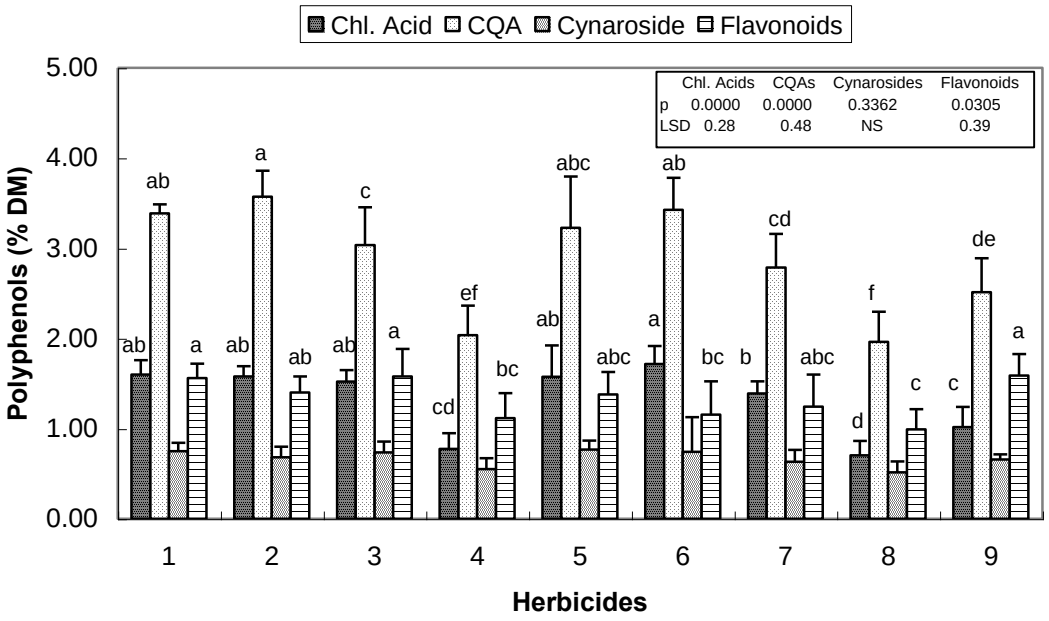


1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Figure 63: Effect of herbicides on polyphenols (% DM) of artichoke in Giessen, 2WAA 1st growth phase 2008 (T = ±SD)

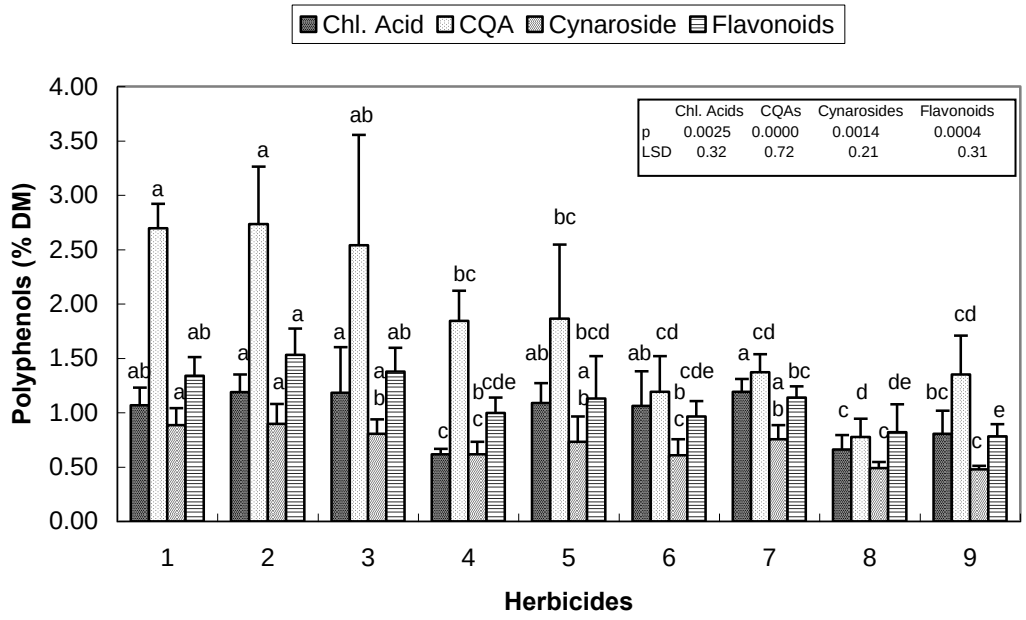
Maximum chlorogenic acids were produced by the application of Prosulfocarb that were statistically at par and followed by that of control, Carfentrazone and Phenmedipham (figure 64). Application of Aclonifen produced minimum contents of chlorogenic acids, which were found to be statistically same with that of application of Pyridate. Application of Clomazone produced chlorogenic acids that were statistically lower than that of application of Aclonifen but were statistically at par with that of Pyridate. As for as caffeoylquinic acids are concerned, maximum concentration of total CQA was observed in case of application of Carfentrazone that were statistically similar and followed by that of Prosulfocarb, control and Quizalofop-P. Minimum contents of CQA were found in case of application of Aclonifen and these were statistically at par with that of application of Pyridate. All other herbicide treatments used in the study showed a range of chlorogenic acids which varied between both maximum and minimum limits. Maximum cynarosides were produced by the application of Quizalofop-P that was slightly higher than that observed by the application of Aclonifen. Cynarosides obtained by other experimental treatments were closer to both Aclonifen (minimum cynarosides) and Quizalofop-P (maximum cynarosides). Total flavonoids in this case were found in case of application of Clomazone, which were statistically at par and followed by that of Phenmedipham, control, Carfentrazone, Quizalofop-P and Rimsulfuron. Application of Aclonifen produced minimum concentration of flavonoids that were statistically at par with the

slightly higher concentration of flavonoids obtained by the application of Phenmedipham, Prosulfocarb and Quizalofop-P.



1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Figure 64: Effect of herbicides on polyphenols (% DM) of artichoke in Giessen, 3WAA 1st growth phase 2008 (T = ±SD)

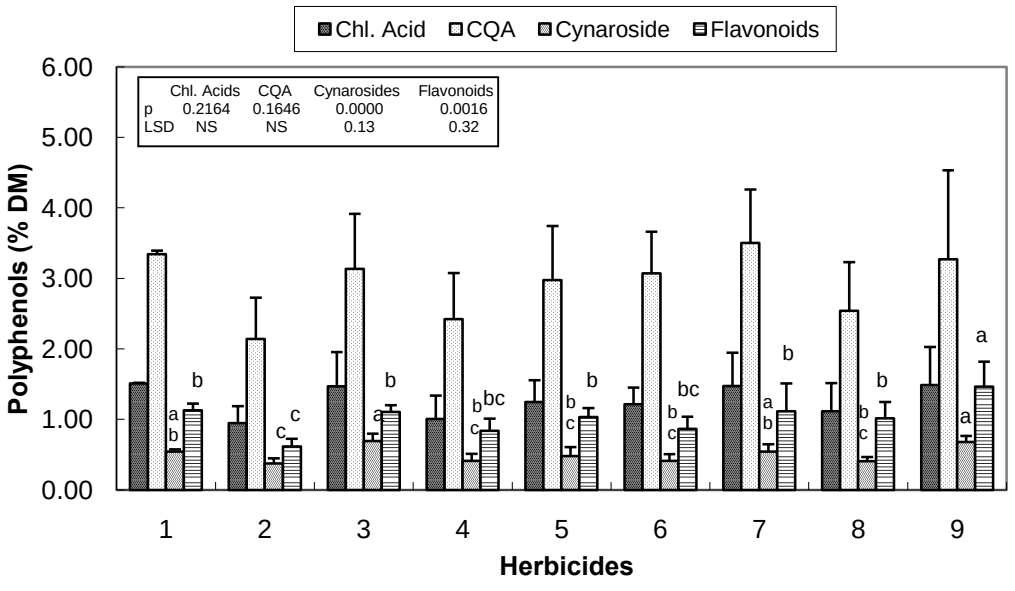


1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Figure 65: Effect of herbicides on polyphenols (% DM) of artichoke in Giessen, 4WAA 1st growth phase 2008 (T = ±SD)

Application of Carfentrazone produced maximum concentration of all polyphenolic compounds detected in study (figure 65). In all the cases these compounds were statistically same with that of control and application of Phenmedipham. Minimum contents of chlorogenic acids and CQA were observed by the application of Aclonifen, which were statistically at par with that of Pyridate, Clomazone for both and with that of Rimsulfuron for CQA. Minimum concentration of cynarosides and flavonoids was observed, where Clomazone was applied as an herbicide. Both these compounds in this case were statistically similar with that of application of Aclonifen, Pyridate and Prosulfocarb.

Chlorogenic acids and CQA showed a non significant response, whereas cynarosides and flavonoids showed a statistically profound response to the herbicidal treatments (see figure 66). Maximum chlorogenic acids were obtained in control, followed by that of the application of Clomazone. Minimum chlorogenic acids in this case were observed by the application of Carfentrazone. Chlorogenic acids obtained by the application of all other herbicides were found to be closer to each other and also with the treatments, where maximum and minimum contents of chlorogenic acids were recorded. As for as CQA are concerned, application of Rimsulfuron produced maximum contents of these compounds that were nearly similar with that of control, Clomazone, Phenmedipham, Prosulfocarb and Quizalofop-P. Minimum CQA were observed by the application of Carfentrazone. For both cynarosides and flavonoids, application of Clomazone produced maximum contents of both these, which were slightly higher for cynarosides but statistically similar with that of control and were statistically different with that of all the other experimental treatments for flavonoids. Both these compounds showed minimum concentration by the application of Carfentrazone that was statistically at par with that of Quizalofop-P, Prosulfocarb and Phenmedipham. Application of Phenmedipham showed a varied response,



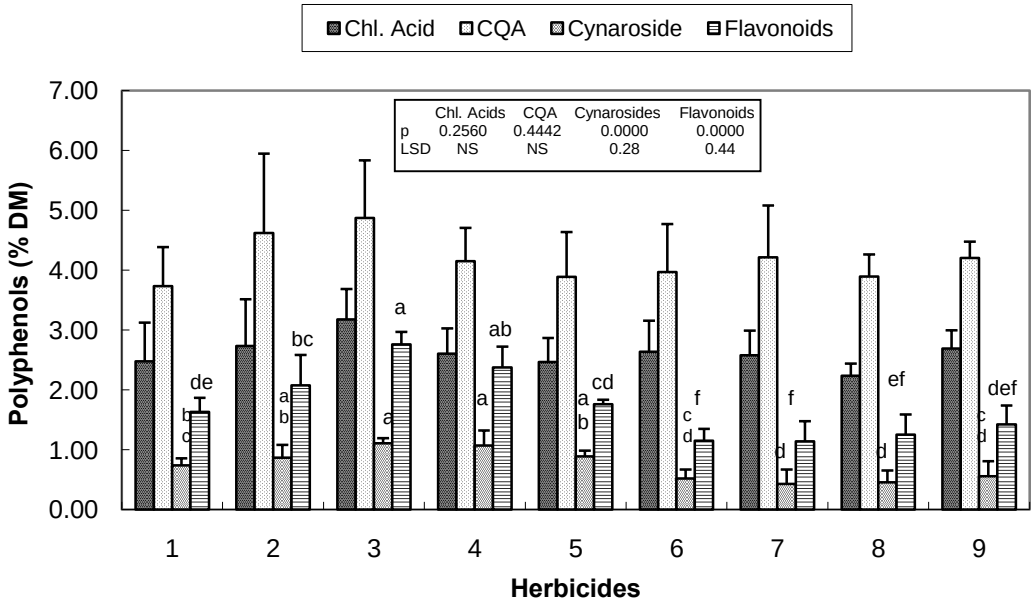
1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Figure 66: Effect of herbicides on polyphenols (% DM) of artichoke in Giessen, 1st cut 2008 (T = ±SD)

where it resulted in the contents of poly phenols that ranged between the maximum and minimum ones.

A close observation of figures 62-66 demonstrates that polyphenols showed an increasing trend towards the harvest, where these show minimum contents at 1 WAA, whereas maximum amount of these compounds was observed at 3 WAA and onwards till the time of first harvest of artichoke crop.

Response of polyphenols to the applied herbicides in second phase of artichoke growth is to be shown in figure 67. Application of Phenmedipham produced maximum contents of all the studied compounds at this time. Application of Aclonifen (nearly same with that of Quizalofop-P and control) produced maximum chlorogenic acids, whereas control produced minimum CQA contents. All other treatments showed similarity with both the maximum and minimum content treatments. Application of Rimsulfuron caused the minimum production of both cynarosides and flavonoids that were slightly lower but statistically at par with that of application of Aclonifen, Clomazone and Prosulfocarb.



1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Figure 67: Effect of herbicides on polyphenols (% DM) of artichoke in Giessen, 1WAA 2nd growth phase 2008 (T = ±SD)

Herbicides used during the course of the study affected all the compounds with the exception of cynarosides, which showed a minor difference in its contents in response to the different herbicidal treatments but these were statistically at par with one another (figure 68). Maximum concentration of chlorogenic acids and CQA was observed in case of the application of Rimsulfuron that was statistically similar with that of Prosulfocarb and Carfentrazone for both compounds and additionally with Aclonifen too for CQA. Minimum amount of both these compounds was detected in case of application of Pyridate, which were slightly higher but significantly same with that of Phenmedipham, Clomazone, Aclonifen and Quizalofop-P for chlorogenic acids and with Quizalofop-P, Clomazone, Aclonifen and Phenmedipham. Other

herbicide treatments used in the study showed a mixed response to these compounds. Maximum flavonoids were observed by the application of Clomazone that were statistically similar and followed by that of application of Rimsulfuron, Aclonifen and Prosulfocarb. Minimum concentration of flavonoids was observed where Carfentrazone was applied as herbicidal treatment and was found statistically similar with that of Quizalofop-P and Pyridate.

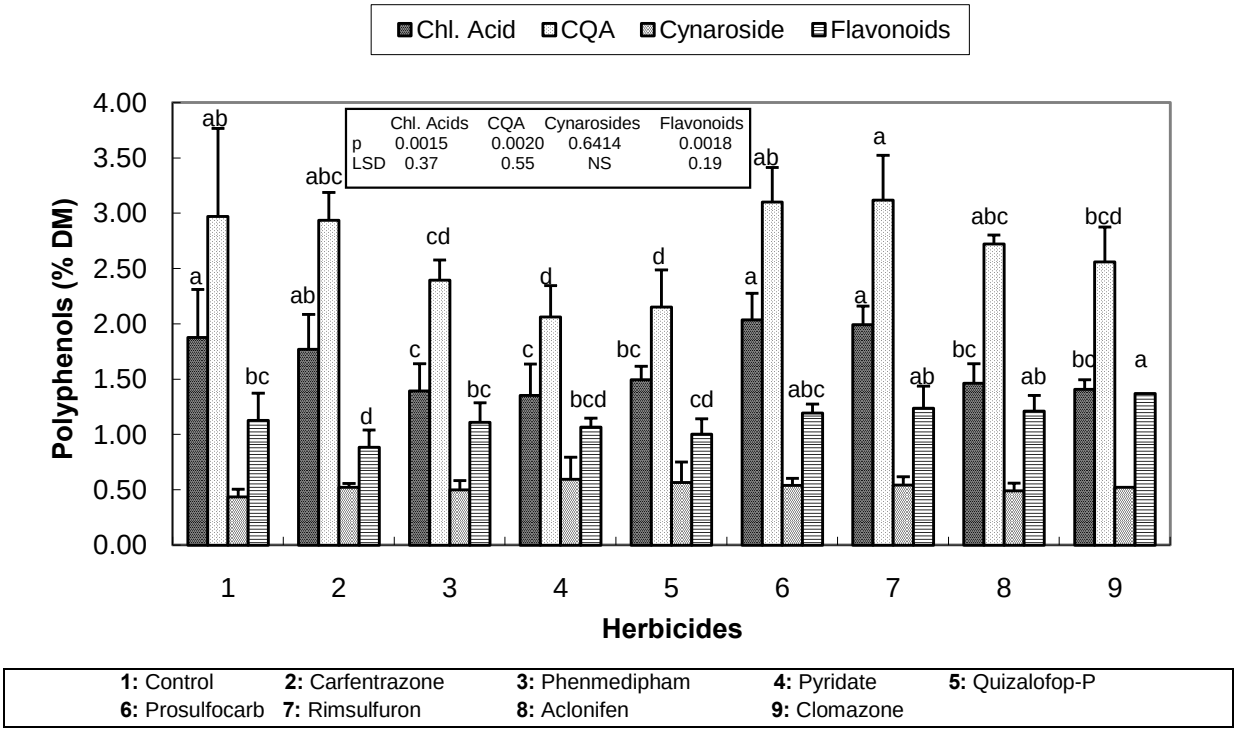
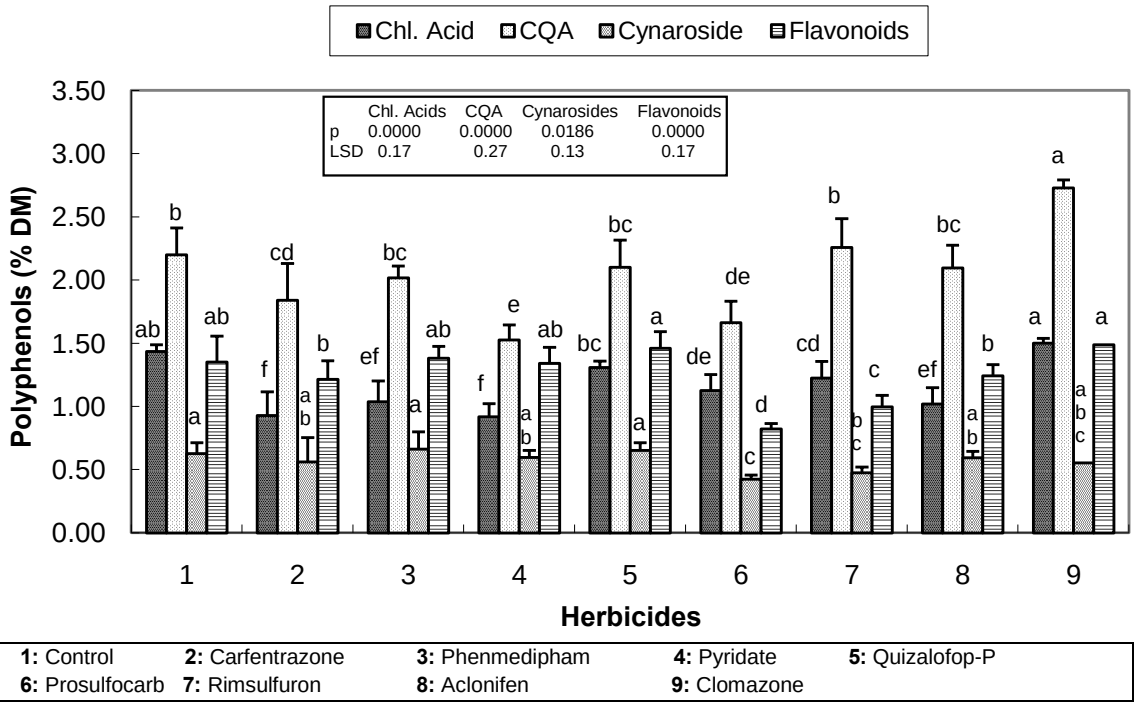


Figure 68: Effect of herbicides on polyphenols (% DM) of artichoke in Giessen, 2WAA 2nd growth phase 2008 (T = ±SD)

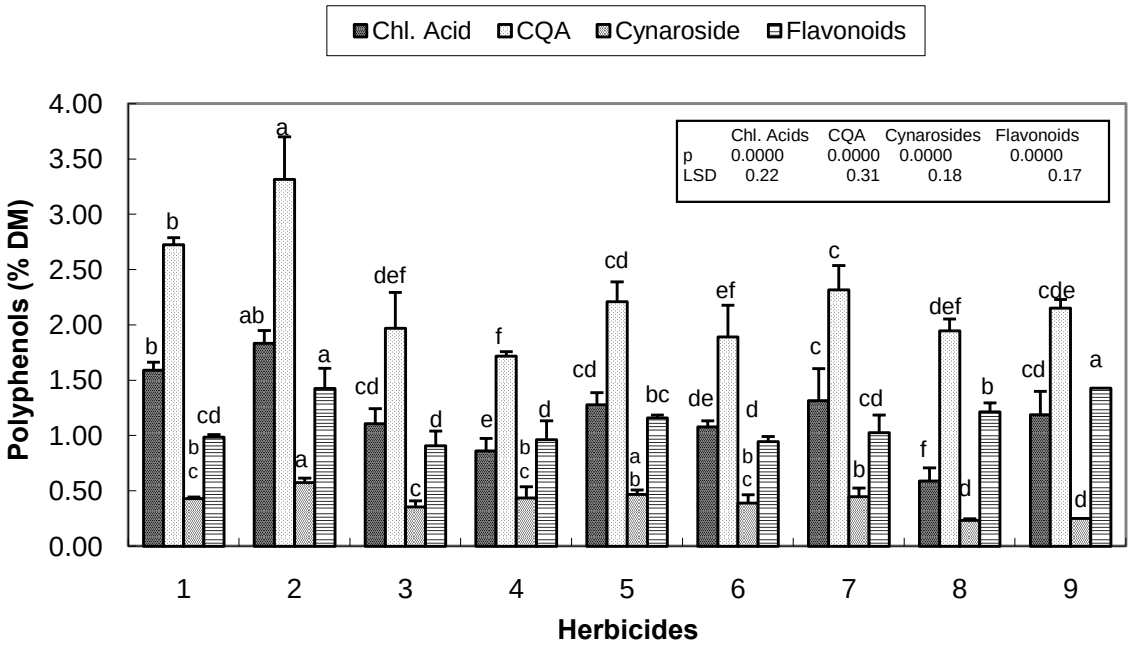
Effect of the herbicidal treatments on the phenolic contents of artichoke leaves, detected at 3 WAA, second growth phase, 2008 reveals a significant response of the polyphenols (figure 69). Maximal chlorogenic acids and CQA were detected in the treatments applied with Clomazone, which were statistically different and higher than that of all other treatments, with the exception that chlorogenic acids obtained at control were statistically at par with the ones at Clomazone. Application of Pyridate produced minimum concentration of both chlorogenic acids and CQA that were slightly lower but statistically at par with that of Carfentrazone, Phenmedipham and Aclonifen for chlorogenic acids and with Prosulfocarb in case of CQA. Application of Phenmedipham showed maximum amount of cynarosides that were a bit higher but significantly similar with that of Quizalofop-P, control, Pyridate, Aclonifen, Carfentrazone and Clomazone. Application of Clomazone resulted in maximum flavonoids that were statistically at par with that of Quizalofop-P, Phenmedipham, Pyridate and control. Minimum concentration of flavonoids was observed in case of application of Prosulfocarb that was statistically lower when compared with that of all other treatments used in the study. Statistically higher concentration of flavonoids was observed in case of application of Rimsulfuron.



1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Figure 69: Effect of herbicides on polyphenols (% DM) of artichoke in Giessen, 3WAA 2nd growth phase 2008 (T = ±SD)

Statistically significant response of polyphenols to the herbicide treatments used during the course of the study is to be seen in figure 70. It was observed that all four compounds showed maximum concentration by the application of Carfentrazone, which were statistically higher than that of all other treatments used in the study with the exception that flavonoids obtained by the application of Clomazone were



1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Figure 70: Effect of herbicides on polyphenols (% DM) of artichoke at in Giessen, 4 WAA 2nd growth phase 2008 (T = ±SD)

statistically at par with those detected by application of Carfentrazone. Minimum concentration of chlorogenic acids was observed in case of application of Aclonifen that was statistically lower than that of application of all other experimental treatments. In case of CQA minimum concentration was observed in case of application of Pyridate, which was statistically at par with that of Prosulfocarb, Phenmedipham and Aclonifen. Minimum cynarosides were observed in case of application of Aclonifen that was statistically at par with that of Clomazone. Application of Phenmedipham showed minimum concentration of flavonoids, which showed statistical similarity with that by the application of Pyridate.

Maximum content of chlorogenic acids was produced by the control and this was statistically similar and followed by that of Prosulfocarb and Carfentrazone (figure 71). Minimum concentration of chlorogenic acids was resulted in the treatment where Clomazone was applied as herbicide and it was statistically at par with that of the application of Aclonifen and Pyridate. As for as CQA are concerned, maximum content of these compounds was observed by the application of Prosulfocarb, which was statistically same with that of the application of Rimsulfuron, Carfentrazone, Quizalofop-P and Phenmedipham. Minimum concentration of CQA was observed in case of application of Aclonifen that was statistically at par with that of control, Pyridate and Clomazone. Carfentrazone and control showed similar and maximum contents of cynarosides in the artichoke leaves detected at second harvest of 2008. Minimum cynarosides were observed by the application of Aclonifen. All other experimental treatments produced cynarosides that, although, were slightly different with one another but were very near to both the treatments where maximum and minimum cynarosides were produced. Application of Quizalofop-P produced maximum concentration of flavonoids, which were statistically at par and followed by that of Prosulfocarb, Aclonifen Clomazone and Rimsulfuron. Minimum flavonoids were observed by the application of Phenmedipham that showed a statistical similarity with that of Carfentrazone, control and Rimsulfuron.

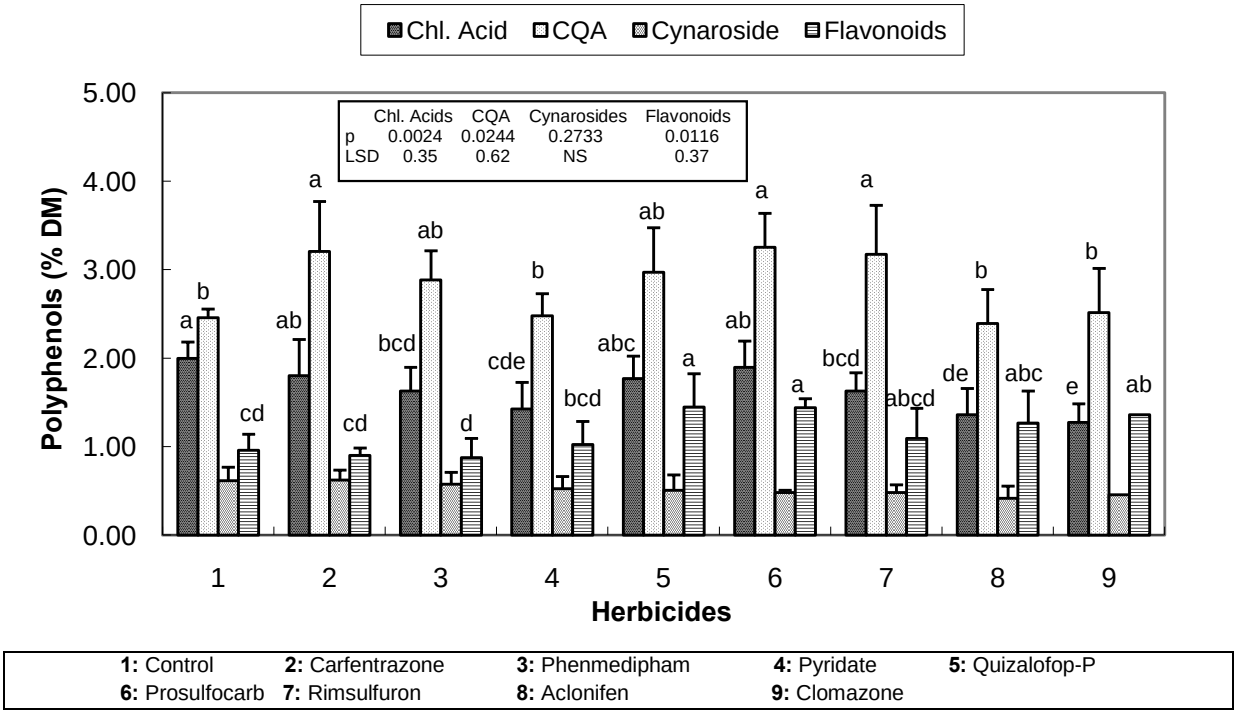


Figure 71: Effect of herbicides on polyphenols (% DM) of artichoke in Giessen, 2nd cut 2008 (T = ±SD)

In contrast to the first growth phase of artichoke, concentration of polyphenols in artichoke leaves decreased with time in the second growth phase. Maximum polyphenols were observed at 1 WAA and these went on decreasing till 3 WAA and showed a constant concentration till 4 WAA. These increased a bit during the later period of growth as is clear from the polyphenol concentration detected at second harvest (figures 67-71).

4. Discussion

4.1 Harvest frequency and plant density

Artichoke is a perennial crop which is able to re-establish its leaves after harvest. This characteristic of the crop enables several cuts or harvests of artichoke leaves within one growing season. Generally artichoke is harvested three times in a growing season in Germany. However under the climate and growing conditions of the executed field experiments in Gross Gerau more than three harvests of artichoke leaves could be achieved. In the executed experiments three systems of harvest frequency were compared: HF 1 - low number of harvests (3 cuts per season), HF 2 - medium number of harvests (5 cuts per season) and HF 3 - high number of harvests (6 cuts per season).

4.1.1 Growth and yield response to harvest frequency and plant density

In 2006 plant height showed a significant response to plant density, whereas in 2007 and 2008 its response to the study factors was non-significant, although the trend with minor differences was same as that of first experimental year. Low plant height observed in plots with high harvest frequencies may be related to the less vegetative growth period of the artichoke crop. The growing period of the leaves in HF 1 – low harvest frequency (95, 40 and 50 days consecutively for first, second and third harvests). Contrarily shorter development periods of the leaves in HF 2 – medium harvest frequency (75 days for first harvest and 30 days each for subsequent harvests) and in HF 3 – high harvest frequency (75 days for first harvest and 21 days each for subsequent harvests). Smaller growth period reduced the duration of photosynthesis as well as water and nutrient uptake of artichoke plants which resulted in smaller plant stand. It may be thought that increasing plant population per unit area increased the intra-specific competition and plants got more height than that of vigour and grew tall.

Plant cover showed a direct relationship with plant density and an inverse relationship with harvest frequency. Higher plant cover in case of highest plant density is due to the higher plant and leaf number per area which induced higher plant cover in total. The differential effect of the study factors on growth and yield components of artichoke confirm the findings of Honermeier et al. (2009), who got similar results in a study conducted in Giessen during the years 2001- 2003. Higher values of the growth parameters of artichoke got at low harvest frequency may be a result of longer vegetative growth period of the crop. Higher values in response to the higher plant density can be related with the fact that although less number of leaves per plant were produced but total number of leaves per unit area were increased and covered more ground area when compared with that of lower plant densities.

Both green and yellow leaves per plant showed the inverse relationship with both plant density and harvest frequency in their specific effects during all three years of experimentation. Less number of green leaves per plant by high plant densities may be the interspecific competition for water, light and nutrient uptake. Less number of yellow leaves per plant at higher harvest frequency is thought to be a result of the shorter growth period of the crop which led to younger green leaves and reduces the formation of older or senescent yellow leaves. A decrease in number of yellow leaves with an increase of plant density might be a result of less light interception by older

leaves causing chlorosis in these leaves. These results confirm the findings of Honermeier and Goettmann (2010) who concluded a decrease in number of leaves of artichoke in response to an increase in plant density. Decrease in both green and yellow leaves with an increase in harvest frequency may be connected with the vegetative growth period of the crop. In case of interaction of harvest frequency and plant density, both green and yellow leaves decreased with an increase in harvest frequency within all the plant densities. In case of medium and high harvest frequency only minor differences among the leaves were observed when compared with that of low harvest frequency. Higher number of green and yellow leaves obtained by an interaction of low harvest frequency and lowest plant density may be due to the cumulative effect of both the factors that on one hand helped the crop to stay longer in the field and on the other hand provided extra space for growth of artichoke.

Dry matter percentage of artichoke leaves was affected significantly during first and last year of experimentation, whereas in 2007 it did not show a significant response to the study factors. Dry matter percentage produced an inverse relationship with harvest frequency. This effect might be a result of the longer development phase of the leaves induced by lower harvest frequency. Longer development of artichoke leaves resulted in an increase in leaf area and size of both leaf veins and leaf blades. Ratio of the leaf vein to leaf blade in low harvest frequency in this case was increased and dry matter components i.e., minerals, fibres compounds and fructanes were concentrated in leaf veins resulting in higher percentage of dry matter in this treatment. It may also be a result of the fact of higher transpiration in the artichoke leaves which resulted in higher dry matter contents of the crop. Interaction of the study factors showed a mixed response to dry matter percentage and same was found for the specific effect of plant density, where percentage of dry matter decreased from lowest plant density towards higher plant density and increased at highest plant density.

Leaf yield on both cut and treatment basis was inversely proportional with harvest frequency in all three years of experimentation. These findings deny the findings the previous study conducted by Matthes and Honermeier (2007), who studied the effect of different harvest frequencies on leaf yield and polyphenol contents of artichoke and concluded that longest growth period of artichoke lead to minimum leaf and dry matter yield in Giessen during 2003-2004. Contradiction in both studies might be a result of difference in the growth phase (period between consecutive harvests), which in present study was higher than that of the previous work done by the above mentioned authors. Reduced harvest frequency with only three harvests per season led to longer leaf development phases which induce higher leaf yields per treatment. It can be concluded that reduced harvest frequency (only three cuts) can be more efficient in comparison with higher number of cuts per season. Additionally harvest costs should be considered which increase with higher number of cuts and reduce the economic efficiency of leaf production of artichoke. These findings are also in line with those of Mauromicale and Ierna (2000) who reported an increase of yield (head weight) with increase in the growth period of artichoke.

No significant differences regarding leaf yield were observed among 8, 12 and 16 plants m^{-2}, but significant lower leaf yield was found in plots with 4 plants m^{-2}, which means that there was a yield elasticity of artichoke reaction on plant densities between 8 and 16 plants m^{-2}. Different number of leaves per plant did not affect the leaf yield within this range of plant density. It leads to the conclusion that artichoke

plants were able to compensate different levels of plant density by adapting to the conditions through an increase or decrease in leaf area or number of leaves. However the lowest plant density of 4 plants m^{-2} led to reduced leaf yield. At this density the artichoke plant population was falling below the minimal density which is necessary of optimal leaf yield formation. At the level of lowest plant density maximal number of leaves per plant (total, green and yellow leaves) was observed. But this increased number of leaves per plant did not compensate the reduced number of plants per m^2 due to lower single leaf area and lower single leaf weight. Both leaf and dry matter yield decreased from the interactions of all plant densities involving low harvest frequency, towards the interactions involving high harvest frequency. In this case both yields per harvest as well as yields per treatment showed the same trend. This trend of reduction in the yield with an increase in harvest frequency might be related to the length of the growth period, as in case of increase in harvest frequency, growth period of the artichoke crop was reduced. The length in growth period enables the artichoke crop for better use of the environmental conditions like soil and air temperature, light intensity and interception that induced photosynthesis and as a result more photosynthates were produced.

A comparison of all three experimental years shows that growth parameters of artichoke with the exception of plant population showed higher values in the first growing year 2006 than that of following years 2007 and 2008. Leaf yield of artichoke, when compared between growing seasons also showed the same response. Different response of the leaf yield and yield components second the previous findings of Honermeier et al. (2009) who studied the influence of different cultivars on leaf yield and phenolic contents of artichoke for three experimental years. The authors reported that leaf yield and polyphenols showed significant response to different cultivars and accessions used in the study. The authors also concluded that concentration of CQA in artichoke leaves was higher in first two experimental years (2001 and 2002) and lower in the third experimental year i.e. 2003. Higher values of the growth and yield parameters obtained in these experiments might be a result of the favourable environmental conditions prevailed during the growth season of artichoke in 2006. During the year 2006 monthly average air temperature increased from April till July and reached maximum in July and then decreased towards October. Higher air temperature might be related to extra sunshine prevailing during this growth phase and increased photosynthesis and a result the growth of the leaves was promoted. In addition to the air temperature, precipitation too favoured the growth of artichoke in the year 2006, where precipitation (513.1 mm) recorded for the growth season of artichoke (April to October) was maximum when compared with that of 2007 (452.4 mm) and 2008 (447.3 mm).

4.1.2 Caffeoylquinic acids and flavonoids in relation to harvest frequency and plant density

Phenolic compounds consist of all aromatic molecules form the simple aromatic amino acids to the most complicated condensed tannins. All these compounds are products of the plant aromatic pathway, which consists of three main sections, namely shikimate, phenylpropanoid and the flavonoid sections. Flavonoids are products of the phenylpropanoid pathway. Phenylalanine ammonia-lyase (PAL) directs carbon from aromatic amino acids to the phenylpropanoids in phenylpropanoid metabolism, which forms cinnamic acid from phenylalanine. This step is interface between the primary (shikimate pathway) and secondary metabolism

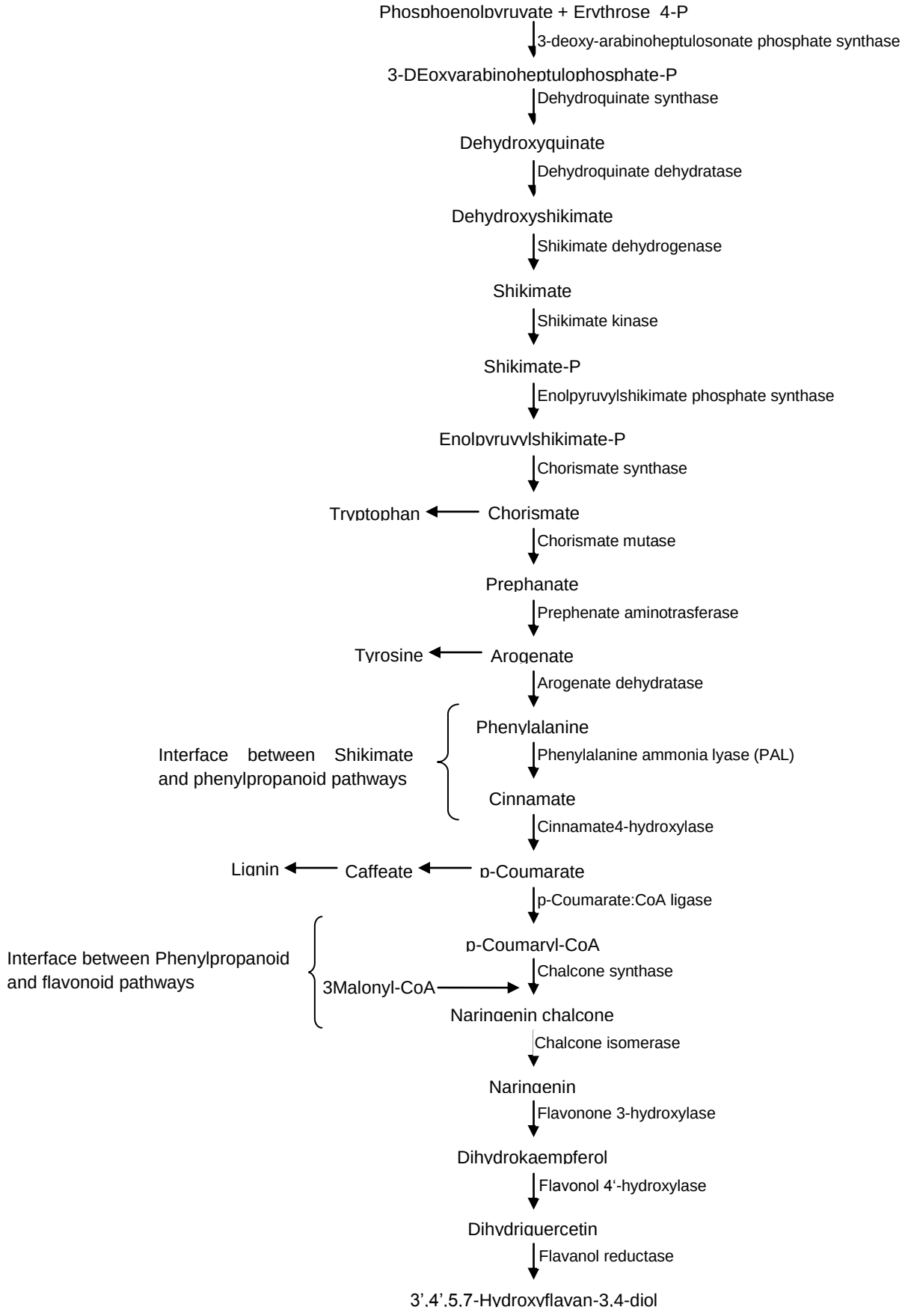


Figure 72: A schematic diagram of the pathway of the phenolic compounds (Hrazdina, 1992)

that leads to various natural products including coumarins, phenolic acid esters, lignins and flavonoids (Figure 72; Hrazdina, 1992). The biosynthesis of flavonoids occurs in organized multi-enzyme complexes, and the transport of flavonoids from the site of synthesis to the final destinations, for example, vacuoles or cell wall, requires specific transferases and membrane transporters (Winkel- Shirley, 2001; Jorgensen et al., 2005). All flavonoids are composed of flavan nucleus (Schijlen et al., 2004; Martens and Mithoefer, 2005). Chalcone synthase is the first enzyme responsible for flavonoid biosynthesis, it condensates three acetate units from malonyl-CoA with *p*-coumaroyl-CoA. The resulting naringenin chalcone (4, 2', 4', and 6'-tetrahydroxychalcone) is rapidly converted to naringenin by the enzyme chalcone isomerase (Fig. 72). These first two enzymes of the flavonoid pathway are found in almost all parts of the plants. However, the enzymes that catalyze the subsequent steps of flavonoid pathway vary from one plant species to the other, giving rise to different flavones, flavonols, anthocyanins and/or proanthocyanidins.

Fratianni et al. (2007) and Rapisarda et al. (1999) concluded that polyphenols of a plant depend on a variety of factors including, genetic and environmental factors. The authors also reported that irrespective of different plant organs, polyphenolic contents in artichoke are higher than that of many other plant species cited in literature. Djeridane et al. (2006) related the abundance of polyphenols in the artichoke a characteristic of the family 'asteraceae', which might be a result of the hard climatic conditions of the usual habitat of members of the family.

It can be supposed that shorter phase of leaf development limited the synthesis of phenolic compounds. It may be supposed that polyphenols increased their concentration in artichoke leaves at rapid rate, which reduced afterwards resulting in accumulation of more amounts of these compounds in the leaves harvested in low harvest frequency. It can be supposed that longer leaf development phase led to higher synthesis and accumulation of CQA compounds in the leaf cells. This effect might be related to the higher rates of UV-B radiation absorption by artichoke leaves. The reason for increased concentration of polyphenols in response to the increased UV-B radiation might be related with the PAL, which is induced by higher UV-B radiation (figure 72). Secondary metabolites like flavonoids also absorb the UV-B wavelengths of light (Karabourniotis et al., 1993; Karabourniotis and Liakopoulos, 2005), in order to counteract to the adverse effects of UV-B radiation artichoke leaves may have developed the cuticular cells which absorb these wavelengths of light and as a result higher concentrations of these compounds are accumulated in these cells. These results are in line with those of Day et al. (1996) who reported that UV-B radiation induces the accumulation of polyphenolic compounds in the epidermis, in the cuticle (Stephanou and Manetas, 1997) and in trichomes (Liakoura et al., 1999). These results also confirm the findings of Yamasaki et al. (2007) who reported that UV-B radiation induces accumulation of polyphenolic compounds initially in trichomes and then in surrounding cell wall. These findings may also be related to the previous findings of Matthes and Honermeier (2007), who reported a significant effect of the vegetative growth period on the polyphenol contents of artichoke leaves. Inverse relationship of these compounds with plant density shows that production of polyphenols was favoured when leaves were provided with wider spaces available for better absorption of light and more aeration during 2008. It can also be supposed that when artichoke plants were grown in wider spaces, it provided the leaves a good chance to grow vertically and the shadowing effect was lower when compared with that of denser populations. As a result of this higher PAR (photosynthetically active radiation) was available to the leaves which resulted in

higher photosynthetic rates and as a result the plant were vigorous and produced more polyphenolic compounds. Leaf blades of artichoke showed higher concentration of polyphenols when compared with that of whole leaves and leaf veins. Leaf veins showed negligible concentration of polyphenols leading to the conclusion that in order to get higher concentration of polyphenols separation of artichoke leaves into leaf blades and veins is necessary. These findings also confirm the findings of Falleh et al. (2008) who reported that polyphenols differ in their concentration in different parts of the artichoke and concluded that maximum polyphenols were concentrated in the leaves of artichoke and were found to be 3.5 and 4.6 times higher than that of seeds and flowers, respectively.

4.2 Effect of Herbicides

Herbicides due to the adverse effect on fluorescence ability of leaves may alter the chlorophyll content of the leaves, or by producing a layer on the leaves may lead to low photosynthetic yield (Miyazawa, 2006). It is an established fact that herbicides may affect plant's physiological state by inhibiting photosynthesis or associated biochemical processes (Krause and Weis, 1984). 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 et al., 1986). Herbicides may enter plants through roots, leaves or both, but in each case they are designed to control weeds 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 (Juneau et al., 2007). Low photosynthetic yield lead to reduced photosynthesis which can induce an inhibition of crop growth and reduction of yields.

4.2.1 Impact of herbicides on growth and yield

Maximum plant height and plant cover observed in control was statistically different and higher than that of all other herbicidal treatments. Reduced number of green leaves per plant was observed in four herbicidal treatments (Phenmedipham, Pyridate, Quizalofop and Prosulfocarb) when compared with that of control, where maximum green leaves per plant were produced. Of these four herbicides Pyridate led to significantly lower number of leaves per plant. Increase in these parameters might be a result of the better environmental conditions available to control as a result of the mechanical weeding, which on one hand removed weeds and on the other provided natural mulch and aeration and also improved the structure of the soil. All these favorable conditions led to the increase of growth rate and as a result maximum values of these parameters were observed in mechanically weeded control plots.

It might be thought that application of herbicides suppressed the growth of artichoke with the most adverse effect of Pyridate. This adverse effect of Pyridate might be a result of the mode of action of this herbicide as it inhibits the photosynthesis at photosystem II (HRAC, 2010) and its effect is favored by high temperature and frequent sunlight conditions (Anonymous, 1991). Maximum number of yellow leaves per plant was observed for application of Phenmedipham and minimum one in control. It can be concluded that herbicides impose a stress on the leaves of artichoke that causes yellowing of the leaves in the crop. Phenmedipham inhibited

the photosynthetic activity of artichoke leaves after its application, which resulted in the yellowing of older leaves and these leaves were not able to recover, although the new leaves emerging after the herbicide application showed no symptoms of herbicide stress.

The control produced maximum leaf yield (t ha^{-1}) during both growth phases of 2006, whereas Pyridate resulted in minimum leaf yield. Leaf yield with the exception of second growth phase was not affected significantly by the herbicides in 2007, although slight differences were observed, where minimum leaf yield was obtained in control. Overall temperature and sunlight prevalence during the second growth phase of artichoke was lesser when compared with that of first growth phase in the year 2007. 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. Minimum leaf yield obtained by the application of Pyridate seconds the finding that the said herbicide had most adverse effect on the artichoke leaves. The adverse effect of Pyridate and Phenmedipham may be related to the mode of action of these herbicides as both these herbicides inhibit photosynthesis at photosystem II. The difference in the adverse effect of these herbicides may be due to different chemical families of these herbicides as Phenmedipham belongs to family 'Phenyl-carbamate' and Pyridate belongs to 'Phenyl-pyridazine' (HRAC, 2010). 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 100 days in comparison to that of 60 days for the second growth phase, 2006, whereas it was noted as 109 and 71 days respectively for 2007. Almost 2 to 2.5 times more precipitation was received during the first growth phase when compared with that of second growth phase and average air temperature during the first growth phase was found to be 17.60 °C which got bit lower during the second growth phase i.e. 14.91 °C in 2006. Second experimental year received 179.4 mm precipitation and 16.63 °C temperature for first growth phase in comparison with 186.5 mm and 12.44 °C of the second one.

Minimum plant height in 2008 was observed by application of Aclonifen. Minimum green leaves per plant were also observed for application of Aclonifen leading to the supposition that it affected the growth of artichoke plants more adversely when compared with other herbicides used during this growth phase. Maximum number of yellow leaves per plant was found by the application of Clomazone, giving an idea that it suppressed the growth during the initial stages of crop and the older leaves were not able to recover against this stress. 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, 2010). 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, 2010). That is the reason why they kill only targeted weeds but additional they can induce stress in the crop.

4.2.2 Herbicides influence on caffeoylquinic acids and flavonoids

Identification and quantification of the polyphenols (caffeoylquinic acids and flavonoids) through HPLC in first two experimental years revealed that application of Pyridate showed lower concentration of CQA and flavonoids. Lower concentration of these compounds might be a result of the stunted crop growth. Application of Pyridate showed severe toxicity symptoms in the form of yellowing of leaves and brown spots on leaf edges. This yellowing of leaves led to lower photosynthetic activity of leaves and hence lower concentrations of phenolic compounds. It may also be hypothesized that these herbicides induced a stress (mechanical injury) to artichoke leaves and certain enzymes of shikimate pathway are reported to be induced by mechanical injury in solanaceae (Dyer et al., 1989). Higher concentrations of these compounds observed in control might be a result of the stress free environment. It might also be supposed that mechanical weeding improved physical properties of the soil, which resulted in high photosynthetic activity and as a result higher concentrations of CQA and flavonoids were observed. Higher concentrations of these compounds shown in the plots treated with Haloxyfop and Prosulfocarb may be a result of non toxic effect of these herbicides. No toxic symptoms were observed in plots treated with these herbicides and there was no yellowing of artichoke leaves. Lush green leaves, thus resulted in higher photosynthetic activity and high comparatively higher concentration of polyphenols.

Minimum concentration of polyphenols in 4 weekly collected samples was observed for Aclonifen treatment, whereas at harvest it was Carfentrazone that showed minimal polyphenols in artichoke in first growth phase. It may lead to the idea that although the adverse effect of Aclonifen did not destroy the crop but affected its phenolic contents and this effect started to appear immediately after the application of the herbicide. A comparison of this growth phase showed that concentration of polyphenols increased from 1 WAA of herbicide till 3 WAA and then decreased marginally towards 4 WAA with maximum polyphenols at final harvest. Minimum concentration of polyphenols during the initial days of growth may be a combined effect of the herbicides and the vegetative growth of the crop. Polyphenols showed an opposite response in second phase of artichoke growth in 2008. Here concentration of polyphenols decreased from 1 WAA till 3 WAA and then increased marginally towards 4 WAA. Polyphenols analyzed at second harvest of artichoke proved to be higher than that of 4 WAA, but lower than that of 1 WAA. The findings of the second growth phase are contrary to the results of first growth phase that vegetative growth period of the crop was the reason for the low concentration of these compounds. A close observation of the environmental factors during both growth phases of artichoke may help to find the reason as average air temperature was 18.1, 21.6, 16.5, 19.8 and 25.0 °C, respectively at 1 DAA, 1 WAA, 2 WAA, 3 WAA and 4 WAA. Whereas in second growth phase it was noted as 10.1, 11.3, 10.2, 13.3 and 7.1 °C, respectively at 1 DAA, 1 WAA, 2 WAA, 3 WAA and 4 WAA. Precipitation received by the artichoke crop during both growth phases had no big differences as it was amounted to be 179.4 mm for first growth phase in comparison

with that of 186.5 mm in second growth phase. These environmental data may lead to the idea that ideal air temperature for the accumulation of polyphenols moves around 15 °C and it needs longer nights when compared with that of day times (Honermeier and Goettmann, 2010). In case of application of individual herbicides, application of Aclonifen resulted in slightly lower values of polyphenols at 1 WAA, whereas in later analyses Pyridate was found to be the herbicide responsible for the lower concentration of polyphenols in artichoke leaves. At second harvest i.e. end of second growth phase minimum polyphenols were observed for herbicidal application of Clomazone. Aclonifen, Clomazone and Pyridate showed their adverse effects on the contents of polyphenols during the second growth phase of artichoke in 2008.

4.2.3 Herbicides and chlorophyll fluorescence

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; Sestak and Stiffel, 1997; 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. Electron transport rate (µmol m^{-2} sec^{-1}) calculated by biochemical methods was also reported to show same dependence on leaf temperature.

First two experimental years i.e. 2006 and 2007 showed the same trend of the response of chlorophyll fluorescence and ETR to the post emergence herbicides used as experimental treatments with the exception that overall values of these parameters during the second growth phase were marginally lower than that of the first ones. Photosynthetic data recorded under direct sunlight showed that Pyridate affected both chlorophyll fluorescence and ETR right after its application as clear form the minimum values obtained at 1 DAA of herbicides in comparison with that of control. These went on decreasing till 2 DAA and then started increasing and reached closer to that of control showing the recovery of the artichoke against the adverse effects of this herbicide. Same trend was shown by the artichoke leaves

when chlorophyll fluorescence was measured under dark adapted conditions. These results confirm the findings of van Rensen (1989), who reported the adverse effect of the herbicide Diuron on plant photosynthesis. The immediate adverse effect of Pyridate may be related to the mode of action of this herbicide, as this herbicide is absorbed by plants through leaves and prevents the production of D1-protein in photosystem II (HRAC, 2010). Action of this herbicide is favored by warm and humid conditions and the adverse effects start to appear from the leaf margins towards the inside of leaves (Anonymous, 1991). At the same time artichoke growth is favored by these environmental conditions. Artichoke crop recovered against this stress with the help of these favorable environmental conditions, which helped it to grow vigorously and produce new leaves. For the reason, it showed chlorophyll fluorescence values in the range comparable to that of control at 3 and 4 WAA. These findings are also supported by a previous finding of Haynes et al. (2000), who studied the effect of Diuron on three species of sea grass and concluded that all studied species showed a rapid fluorescence response to the applied herbicide. Genty et al. (1989) reported the measurement of ETR at light saturation (by the use of chlorophyll fluorescence measurement system) for the investigation of photosynthetic capacity.

Toxicity measurements recorded weekly after the application of herbicides confirm the adverse effects of different herbicides imposed on the artichoke leaves. These measurements showed that there were no toxicity symptoms on artichoke leaves at 1 DAA, appeared at 1WAA for Pyridate and went on increasing till 2 WAA for first growth phase and then started decreasing showing the recovery process of the crop. During the second growth phase this adverse effect started decreasing from 2 WAA and it was near to that of control in both growth phases at 4 WAA, where data were recorded for the last time. Data regarding visible toxic symptoms confirm that Pyridate affected the artichoke leaves most adversely. These symptoms were observed in the form of brown spots particularly on older leave of the crop and these started to appear from the leaf margins towards the inner side of leaves.

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 (Anonymous, 2001). 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).

Field (1983) and Hirose and Wagner (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. Chlorophyll fluorescence measured under direct sunlight conditions in 2008 showed that Clomazone affected chlorophyll fluorescence right after its application (1 DAA). This effect went on increasing and was followed by adverse effects of Aclonifen and Rimsulfuron, which showed their effect at 1 WAA. The adverse effect of all herbicides on chlorophyll fluorescence started decreasing from 3 WAA and it was near to normal (in comparison with that of control) at 4 WAA. ETR too showed the same response to the applied herbicides when measured under the light adapted conditions of Giessen. 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 ETR were affected to a certain extent. Pyridate showed the adverse effect on chlorophyll fluorescence measured under direct dark adapted conditions at 1 DAA. Aclonifen showed the adverse effect at 1 WAA followed by that of Clomazone and Pyridate and this stress was recovered by artichoke through 2 WAA towards 4 WAA. 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.

Toxicity measurements showed that visual toxicity symptoms started to appear at 1 WAA and were worst for Clomazone followed by that of Pyridate, Aclonifen, Rimsulfuron and Carfentrazone. This adverse effect went on increasing till 3 WAA and then decreased a bit, but there was no complete recovery by the crop during first growth phase in 2008. Figure 73 shows the comparison of the adverse effects of the two of most toxic herbicides along with control for second growth phase, 2008 in Giessen. This toxic effect was worst during the second growth phase of artichoke growth and went on increasing till 4 WAA where data were recorded for the last time. The adverse effect of the herbicides may be related to their modes of action, where Clomazone and Aclonifen inhibit the biosynthesis of carotenoids (HRAC, 2010),

Control **Aclonifen** **Clomazone**

Figure 73: Comparison of the control with two most adverse herbicides in the form of visible toxicity symptoms at 4 WAA during 2nd growth phase, 2008 in Giessen.

which are coloring pigments and their deficiency causes yellowing of leaves which in adverse conditions may lead to the chlorosis of the leaves. Pyridate inhibits the photosynthesis directly at photosystem II (HRAC, 2010) and thus suppresses the crop growth. All these herbicides used during the course of the study are of selective nature, that is why these herbicides do not kill artichoke crop and eradicate only target weeds, even then cause a temporary hazard to the growth of the crop which in certain cases may prevail till the completion of the growth phase.

4.3 Concluding Remarks

Depending on the results of the field experiments conducted from 2006 to 2008 it can be concluded that the traditional harvest frequency (3 harvests) in combination with 8-12 plants m^{-2} is economical and beneficial under the environmental conditions of Germany. Depending on the results obtained, it can also be suggested that maximum polyphenolic compounds (CQA and flavonoids) are concentrated in leaf blades, whereas leaf veins contain negligible amount of these compounds. For that reason, mechanical separation of leaves in blades and veins direct after harvest will result in easier extraction and higher outcome of these compounds.

It can also be concluded that different groups of herbicides impose a stress on the non target plants (artichoke in the study) that differs in its intensity and the crop recovers against it depending on the severity of the stress in different times. It can be concluded that herbicide stress can be identified through photosynthetic measurements made in the form of chlorophyll fluorescence and electron transport rate on one hand and by the quantification of caffeoylquinic acids and flavonoids on the other.

A lot of work is needed to be done in order to study the exact localization of these compounds in the leaves of artichoke and is recommended at the end of this study. Study relating the herbicidal residues in artichoke and their effect on polyphenols is also recommended. It is also recommended that adverse effects of the herbicides should be studied more frequently, particularly immediately after the application of the herbicides.

5. Summary

Artichoke is a traditional vegetable plant grown and used in subtropical and Mediterranean regions. Artichoke leaves and flower buds contain high concentration of polyphenols, which are attributed to nutritional and pharmacological effects. These compounds include caffeoylquinic acids (chlorogenic acid, caffeic acid and cynarin), flavonoids (scolymoside, cynaroside and luteolin) and sesquiterpene lactones.

Although, artichoke has enormous importance in the Mediterranean region, certain climatic limitations discourage its cultivation in Central Europe. Yet, in Germany it is cultivated as a commercial annual leaf crop. Cultivation in Germany is focused on the production of rosette leaves which contain high contents of polyphenols that are used for dyspeptic complaints.

Contrary to the vegetable production of artichoke, only a few research work on its importance as leaf crop has been done. This research area needs a focus on the influence of the environmental conditions and agronomic factors on yield and polyphenols contents of artichoke leaves under field conditions in Germany.

Keeping these points into consideration, research was conducted at University Giessen to study the effect of plant density (4, 8, 12 and 16 plants m^{-2}) and harvest frequency (LHF- low harvest frequency with 3 harvests, MHF- medium harvest frequency with 5 harvests and HHF- high harvest frequency with 6 harvests per growing season) on plant growth, yield and polyphenols of artichoke leaves. Simultaneously, a second experiment to study the impact of different post-emergence herbicides (Haloxyfop, Phenmedipham, Pyridate, Quizalofop-P, Prosulfocarb, Carfentrazone, Rimsulfuron, Aclonifen and Clomazone) on leaf yield and the concentration of polyphenols (CQA and flavonoids) was conducted. The experiments were conducted at experimental research stations Gross-Gerau and Giessen, respectively. Cultivar Gobbo di Nizza was sown manually in 75 cm apart rows with 25 cm intra-row distance at 2 cm soil depth. The field trials were designed in RCBD in factorial arrangement (plant density x harvest frequency) and one factor RCBD (herbicides) with 4 replications. Obtained results were analyzed statistically by the PIAF (Programm Information Auswertung Feldversuche - Statistical program for evaluation of field trials).

On the basis of the results obtained in three experimental years, it was found that LHF (3 harvests per growth season) in combination with higher plant densities (12-16 plants m^{-2}) led to best growth and yield parameters (plant height, leaves per unit area, leaf yield) and highest concentration of polyphenols. High harvest frequency (6 harvests per growth seasons) led to lower leaf yield and lower concentration of caffeoylquinic acids and flavonoids in the leaves. Additionally differential response of the interaction of plant density x harvest frequency to some yield parameters was also observed. These differences in growth and yield parameters may be attributed to the specific growth conditions (air temperature, light interception, day length) during leaf formation of artichoke. Plant densities lower than 8 plants m^{-2} failed to compensate the loss in leaf yield by producing more leaves as that of higher plant densities.

Post-emergence application of Pyridate, Aclonifen and Clomazone resulted in reduction of leaf yield in both growth phases of artichoke. Chlorophyll fluorescence data determined that Pyridate reduced chlorophyll fluorescence and electron

transport rate in artichoke leaves significantly, showing a stress to the plants. Phenmedipham, Aclonifen and Clomazone also showed the same negative effects after their application to artichoke leaves. These negative effects of the post emergence herbicides used in the study appeared at 1 DAA (days after application) to maximum of 1 WAA (week after application). The physiological symptoms of stress measured in the form of chlorophyll fluorescence and ETR were not visible at 4 WAA or later. Only Pyridate and Aclonifen showed some adverse effects on the concentration of CQA (up to 1 WAA) in artichoke leaves. CQA and flavonoids determined at harvest of artichoke leaves did not show any adverse effects of the post emergence herbicides used in the research project. Recovery of artichoke against he adverse effects of herbicides may be attributed to the regeneration and formation of new leaves.

6. Zusammenfassung

Die Artischocke ist eine traditionelle Gemüsepflanze, die vor allem in den subtropischen und mediterranen Regionen angebaut und genutzt wird. In den Blütenknospen und Blättern der Artischocke sind relativ hohe Gehalte an sekundären Pflanzenstoffen enthalten, denen positive ernährungsphysiologische und pharmakologische Effekte zugesprochen werden. Zu diesen Verbindungen zählen Caffeoylchinasäuren (Chlorogensäure, Cynarin und Kaffeesäure), Flavonoide (Scolymosid, Cynarosid und Luteolin) und Sesquiterpenlacone ().

Während die Artischocke in den mediterranen Regionen eine vergleichsweise große Bedeutung besitzt, ist ihr Anbau in Mitteleuropa aus klimatischen Gründen nur begrenzt möglich. Trotzdem konnte auch in Deutschland ein kommerzieller Anbau der Artischocke als einjährige Blattpflanze etabliert werden. Das Interesse richtet sich hierbei vor allem auf die Gewinnung von Rosettenblättern zur Herstellung von Trockenextrakten mit hohem Anteil an Caffeoylchinasäuren (CCS), denen eine Wirksamkeit bei der Behandlung dyspeptischer Beschwerden zugesprochen wird.

Im Gegensatz zur Forschung mit Gemüse-Artischocken wurden bislang nur wenige Untersuchungen zur Optimierung der Blattgewinnung der Artischocke durchgeführt. Von Interesse ist hierbei vor allem die Klärung des Einflusses der Wachstumsbedingungen und agronomischer Faktoren auf den Blattertrag und die Konzentration an Caffeoylchinasäuren in den Blättern der Artischocke, die unter Feldbedingungen in Deutschland angebaut wird.

Aus diesem Grund wurden in den Jahren 2006 bis 2008 an der Universität Gießen Feldversuche durchgeführt, die den Einfluss unterschiedlicher Pflanzendichten (4, 8, 12 und 16 Pflanzen m^{-2}) und der Schnittfrequenz der Blätter (niedrige Schnittfrequenz mit 3 Ernten, mittlere Schnittfrequenz mit 5 Ernten und hohe Schnittfrequenz mit 6 Ernten pro Jahr) auf das Pflanzenwachstum, den Blattertrag und die Konzentration an Caffeoylchinasäuren untersuchen sollten. Daneben wurde im gleichen Zeitraum ein Herbizidversuch durchgeführt, in dem der Einfluss von verschiedenen Herbiziden (Wirkstoffe: Haloxyfop, Phenmedipham, Pyridat, Quizalofop-P, Prosulfocarb, Carfentrazone, Rimsulfuron, Aclonifen und Clomazon) auf den Blattertrag und die Konzentration an Caffeoylchinasäuren (CCS) analysiert wurde. Die Experimente wurden in den Versuchsstationen Groß-Gerau (Pflanzendichte-Erntefrequenz-Versuche) bzw. Gießen (Herbizidversuche) mit der Sorte Gobbo di Nizza (2 cm manuelle Ablagetiefe, 75 cm Reihenweite, 25 cm Pflanzabstand) in Parzellen (3,0 x 7,0 m^2) angelegt. Die Feldversuche waren als zweifaktorielle Blockanlagen (Pflanzendichte x Schnittfrequenz) bzw. einfaktorielle Blockanlagen (Herbizide) mit 4 Wiederholungen konzipiert. Die statistische Auswertung der Ergebnisse erfolgte mit dem Programm PIAF (Programm Information, Auswertung, Feldversuche).

Es wurde festgestellt, dass im Mittel der drei Versuchsjahre mit der niedrigsten Schnittfrequenz (3 Ernten pro Jahr) in Kombination mit höheren Pflanzendichten (12-16 Pflanzen m^{-2}) die günstigsten Ertragsparameter (Blattzahl pro m^2, Blattertrag, Pflanzenhöhe) und die höchsten Gehalte CCS-Verbindungen in der Artischocke erzielt wurden. Eine hohe Schnittfrequenz von 6 Ernten pro Jahr führte dagegen zu geringeren Blatterträgen und niedrigeren CCS- und Flavonoidgehalten in den Blättern. Zum Teil wurde eine Wechselwirkung Pflanzendichte x Schnittfrequenz bezüglich der gemessenen Ertragsparameter und der CCS-Gehalte in den Blättern beobachtet. So waren die Effekte der unterschiedlichen Pflanzendichten zum Teil nur bei niedriger Ernte- bzw. Schnittfrequenz zu beobachten. Die Ursachen für den Effekt der Ernte- bzw. Schnittfrequenz werden auf die unterschiedliche Entwicklungsdauer der Blätter in Kombination mit den spezifischen Wachstumsbedingungen (Lufttemperatur, Tageslänge, Lichtangebot) während der Blattbildungsphase zurückgeführt. Niedrige Pflanzendichten von weniger als 8 Pflanzen m^{-2} können von der Artischocke durch eine höhere Blattzahl pro Pflanze offenbar nicht mehr

kompensiert werden, um den gleichen Blattertrag pro Fläche zu erhalten wie mit höheren Pflanzendichten.

In den Herbizidversuchen (je zwei Aufwüchse bzw. Blatternten pro Jahr) wurden mit den Wirkstoffen Pyridate, Aclonifen und Clomazon im Ergebnis einer Nachauflaufbehandlung zum Teil signifikante Wachstumsdepressionen und Mindererträge in den Artischocken beobachtet. Mit Hilfe der durchgeführten Chlorophyllfluoreszenz-Messungen wurde unmittelbar nach der Applikation von Pyridat eine deutliche Verminderung der Elektronentransportrate und der Chlorophyllfluoreszenz in den Blättern festgestellt, die als Stressreaktion der Pflanze gewertet werden. Ähnliche Negativeffekte auf die Chlorophyllfluoreszenz wurden auch bei den Herbizidwirkstoffen Phenmedipham, Aclonifen und Clomazon beobachtet. Die Stressreaktion der Pflanze war bereits einen Tag nach der Herbizidapplikation bis maximal 1 Woche nach der Behandlung messbar. Nach etwa 4 Wochen waren die physiologischen Stress-Symptome, gemessen an der Chlorophyllfluoreszenz und Elektronentransportrate in den Blättern, nicht mehr festzustellen, was mit der Regeneration und Neubildung von Blättern der Artischockenpflanze begründet wird. Die Gehalte an Caffeoylchinasäuren in den Blättern wurden durch die Herbizidapplikation nur durch Pyridate und Aclonifen kurzzeitig (bis 1 Woche nach der Behandlung) beeinflusst. Bei sachgemäßer Anwendung von Herbiziden ist zum Zeitpunkt der Blatternte der Artischocke ist nicht mit einer durch die Herbizidapplikation induzierten Verminderung der Gehalte an Caffeoylchinasäuren in den Blättern zu rechnen.

7. References

Anonymous. 1991. Pyridate: Decision document E91-01. Pest Management Regulatory Agency. Health Canada. Ottawa, Ontario.

Anonymous. 2001. Pyridate Evaluation Report by European Commission - Directorate General of Health & Consumer Protection.

Anonymous. 2002. WHO traditional medicinal report 2002-2005. World Health Organization, Geneva

Anonymous. 2007. Clinical uses of artichoke leaf extract. Am. J. Health-Syst. Pharm. 64, 1904-1909.

Benlloch-Gonzalez, M., J. M. Fournier, J. Ramos and M. Benlloch. 2005. Strategies underlying salt tolerance in halophytes are present in *Cynara cardunculus*. Plant Sci. 168, 635–659.

Bianco, V. V. 2005. Present situation and future potential of artichoke in the Mediterranean basin. Acta Hort. 681, 39–55.

Bilger, W. and O. Bjorkman. 1990. Role of xanthophylls cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Photosynth. Res. 25, 173–185.

Bolhar-Nordenkampf, H. R., S. P. Long, N. R. Baker, G. Oquist, U. Schreiber and E. G. Lechner. 1989. Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. Functional Ecol. 3, 497-514.

Brand, N and F. Weschta. 1991. Die analytische Bewertung der Artischocke und ihrer Praeparate. Zeitschrift fuer Phytotherapie, 12, 15-21.

Bundy, R., A. F. Walker, R. W. Middleton, G. Marakis and J. C. L. Booth. 2004. Artichoke leaf extract reduces symptoms of irritable bowl syndrome and improves quality of life in otherwise healthy volunteers suffering from concomitant dyspepsia: A subset analysis. The J. of Altern. Compl. Med. 10 (4), 667-669.

Buschmann, C. 1995. Variation of the quenching of chlorophyll fluorescence under different intensities of the actinic light in wild type plants of tobacco and in an *Aurea* mutant deficient of light-harvesting-complex. J. Plant Physiol. 145, 245–252.

Dalianis, C., C. Panoutsou and N. Dercas. 1996. Spanish thistle artichoke (*C. cardunculus* L.) under Greek conditions. In: Chartier, P., G. L. Ferrero and U. M. Henius et al. (Eds.), Biomass for energy and environment (Proceedings of the 9th European Bioenergy Conference, 24-27 June, Copenhagen, Denmark). Elsevier Science, Oxford. 663–668.

Damato, G., M. A. Cascarano and L. Casciaro. 2006. First results of some cultural practices on yield and quality of artichoke “seed”. Acta Hort. 730, 201-209

Damato, G., V. V. Bianco and N. Laterza. 1994. First results of plant density and nitrogen rate on yield and quality of Florence fennel (*Foeniculum vulgare* Mill. var. *azoricum* Thell.) ‘seeds’. Acta Hort. 362, 67-73.

Day, T. A., B. W. Howells and C. T. Ruhland. 1996. Changes in growth and pigment concentrations with leaf age in pea under modulated UV-B radiation field treatments. Plant Cell Environ. 19, 101-108.

Djeridane, M., B. Yousfi, D. Nadjemi, P. Boutassouna and N. Stocker. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem. 97, 654–660.

Durigan-Marcel, E. B., J. C. Durigan and M. Gustavo. 2005. Selectivity of the herbicide flazasulfuron applied after postemergence in sugarcane (*Saccharum* spp. L.) crop. J. Environ. Sci. Health. B40, 177-180.

Dyer, W. E., J. M. Henstrand, A. K. Handa and K. M. Hermann. 1989. Wounding induces the first enzyme of the shikimate pathway in solanaceae. Proc. Natl. Acad. Sci. USA. 86, 7370-7373.

El Jay, A., J. M. Ducruet, J. C. Duval and J. P. Pelletier. 1997. A high sensitivity chlorophyll fluorescence assay for monitoring herbicide inhibition of photosystem II in the chlorophyte *Selenastrum capricornutum*: comparison with effect on cell growth. Arch in Hydrobiol. 140, 273–286.

Elia, A., F. Paolicelli and V. V. Bianco. 1991. Effect of sowing date, plant density and nitrogen fertilizer on artichoke (*Cynara scolymus* L.) preliminary results. Adv. Hort. Sci. 5, 119-122.

Falleh, H., R. Ksouri, K. Chaieb, N. Karray-Bouraoui, N. Trabelsi, M. Boulaaba and C. Abdelly. 2008. Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. C. R. Biologies. 331, 372–379.

FAO.2009. http//www.fao.org

Fernandez, J., 1992a. Production and utilisation of *C. Cardunculus* L. biomass for energy, paper-pulp and food industry. In: Grassi, G., A. Colina and H. Zibetta (Eds.), Biomass for energy, industry and environment (Proceedings of the 6th E. C. Conference, 22–26 April, Athens, Greece). Elsevier Science, London. 312–316.

Fernandez, J., 1992b. Final report. Project JOUB 0030 Production and utilisation of *Cynara cardunculus* L. biomass for energy, paper-pulp and food industry. May 1990–August 1992. EEC, DG XII, F, Atlanta, p. 4.

Field, C. 1983. Allocating leaf nitrogen for the maximization of carbon gain – leaf age as a control on the allocation program. Oecologia. 56, 341-347.

Frank, R. and L. Logan. 1988. Pesticides and industrial chemical residues at the mouth of the Grand, Saugeen and Thames Rivers, Ontario, Canada 1981-85. Arch. Environ. Contam. Toxicol. 17, 741- 754.

Fratianni, F., M. Tucci. M. De Palma, R. Pepe and F. Nazzaro. 2007. Polyphenolic composition in different parts of some cultivars of globe artichoke (*Cynara cardunculus* L. var. s*colymus* (L.) Fiori). Food Chem. 104, 1282-1286.

Gaillardon, P., J. C. Guichaoua, J. Gasquez and R. Scalla. 1989. Absorption, translocation and metabolism of pyridate in a tolerant crop (Zea mays) and two susceptible weeds (*Polygonum lapathifolium* L. and *Chenopodium album* L.). Weed Res. 29, 45-51.

Gebhardt, R. 1997. Antioxidative and protective properties of extract from leaves of the artichoke (*Cynara scolymus* L.) against hydroperoxide-induced oxidative stress in cultured rat hepatocytes. Toxicol. Appl. Pharmacol. 144, 279-286.

Genty, B., J. M. Brantais and N. R. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochem. Biophys. Acta. 990, 87-92.

Gominho, J., J. Fernandez and H. Pereira. 2001. *Cynara cardunculus* L. – a new fibre crop for pulp and paper production. Ind. Crops Prod. 13, 1-10.

Grammelis, P., A. Malliopoulou, P. Basinas and N. G. Danalatos. 2008. Cultivation and Characterization of *Cynara Cardunculus* for Solid Bio fuels Production in the Mediterranean Region. Int. J. Mol. Sci. 9, 1241-1258.

Grancai, D., M. Nagy, V. Suchy and P. Novomesky. 1994. Cynarin from the fresh flower buds of *Cynara cardunculus*. Fitoterapia. 65, 282.

Gray, D., J. R. A. Steckel and J. A. Ward. 1983. Studies on carrot seed production. Effects of plant density on yield and components of yield. Hort. Sci. 58, 83-90.

Halliwell, B., R. Aeschbach, J. Loliger and O. I. Aruoma. 1995. The characterization of antioxidants. Food Chem. Toxicol. 33, 601-617.

Hanelt, P. 2001. Mansfeld's Encyclopaedia of Agricultural and Horticultural crops. 2040-2041.

Hanhineva, K. 2008. Metabolic Engineering of Phenolic Biosynthesis Pathway and Metabolite Profiling of Strawberry (*Fragaria x ananassa*) Kuopio Univ. Publ. C. Nat. and Environ. Sci. 231, 1-89.

Haynes, D., P. Ralph, J. Prange, and B. Dennison. 2000. The Impact of the herbicide diuron on photosynthesis in three species of tropical seagrass. Marine Pollution Bulletin. 41 (7-12), 288-293.

Hendrickson, L., M. C. Ball, J. T. Wood, W. S. Chow and R. T. Furbank. 2004. Low temperature effects on photosynthesis and growth of grapevine. Plant Cell Environ. 27 (7), 795–809.

Hirose, T. and M. J. A. Werger. 1987. Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. Oecologia. 72, 520-526.

Honermeier, B. and S. Goettmann. 2010. Leaf yields and concentrations of polyphenolic compounds in artichoke (*Cynara cardunculus* L.) affected by different row spacing and plant densities. J. fuer Kulturpflanzen, 62 (11), 393-401.

Honermeier, B., T. Schaefer and S. Goettmann. 2009. Leaf yields and concentrations of chemical compounds of different cultivars of artichoke (*Cynara cardunculus* L.) – results from field experiments of the site Giessen. J. Med. Spice Plants. 14 (2), 76-83.

HRAC. 2010. The world of herbicides poster. According to HRAC (Herbicide Resistance Action Committee, USA) classification on mode of action (http://www.hracglobal.com/).

Hrazdina, G. 1990. Biosynthesis of flavonoids. In: Plant polyphenols. Ed. Hemingway, R. W. and P. E. Laks. Plenum Press, NY. 61-72

Jorgensen, K., A. V. Rasmussen, M. Morant, A. H. Nielsen, N. Bjarnholt, M. Zagrobelny, S. Bak and B. L. Moller. 2005. Metabolon formation and metabolic channeling in the biosynthesis of plant natural products. Curr. Opin Plant Biol 8, 280-291.

Juneau, P., B. Qiu and C. P. Debolis. 2007. Use of chlorophyll fluorescence as a tool for determination of herbicide toxic effect. Toxicol. Enivron. Chem. 89 (4), 609-625.

Juneau, P., B. R. Green and P. H. Harrison. 2005. Simulation of Pulse- Amplitude-Modulated (PAM) fluorescence: Limitations of some PAM-parameters in studying environmental stress effects. Photosynthetica (Prague). 43, 70–78.

Karabourniotis, G. and G. Liakopoulos. 2005. Phenolic compounds in plant cuticles: Physiological and ecological aspects. Adv. Pl. Phys. 8, 33-47.

Karabourniotis, G., A. Kyparissis and Y. Manetas. 1993. Leaf hairs of *Olea europaea* protect underlying tissues against ultraviolet-B radiation damage. Environ. Exp. Bot. 33 (3), 341-345.

Karlen, D. L., M. J. Kasperbauer and J. P. Zublena. 1987. Row-spacing effects on corn in the southeastern U. S. J. Appl. Agric. Res. 2 (2), 65-73.

Kitajima, M. and W. L. Butler. 1975. Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. Biochim. Biophys. Acta, 376, 105–115.

Koike, T. 1990. Autumn coloring, photosynthetic performance and leaf development of deciduous broad-leaved trees in relation to forest succession. Tree Physiol. 7, 21-32.

Kraft, K. 1997. Artichoke leaf extract-recent findings reflecting effects on lipid metabolism, liver, and gastrointestinal tracts. Phytomedicine. 4 (4), 369-378.

Kramer, M., G. Johnson, O. Kiirats and G. E. Edwards. 2004. New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. Photosynth. Res. 79, 209–218.

Krause, G. H. and E. Weis. 1984. Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. Photosynthesis Research. 5, 139-157.

Krause, G. H. and E. Weis. 1991. Chlorophyll fluorescence and photosynthesis: The basics. Ann. Rev. Plant Physiol. Plant Mol. Biol. 42, 313–349.

Krizkova, L., P. Mucaji, M. Nagy and J. Krajcovic. 2004. Triterpenoid cynarasaponins from *Cynara cardunculus* L. reduce chemically induced mutagenesis in vitro, Phytomedicine. 11, 673–678.

Lavorel, J. and A. L. Etienne. 1977. In vivo chlorophyll fluorescence. In: Barber, J. (Ed.), Primary Processes of Photosynthesis. Elsevier /North-Holland Biomedical Press, Amsterdam, 203–268.

Liakoura, V., S. Stavrianakou, G. Liakopoulos, G. Karabourniotis and Y. Manetas. 1999. Effects of UV-B radiation on *Olea europaea*: comparisons between green house and field experiment. Tree Physiol. 19, 905-908.

Lichtenthaler, H. K. and U. Rinderle. 1988. The role of chlorophyll fluorescence in the detection of stress conditions in plants. CRC - Crit. Rev. Anal. Chem. - 19 [Suppl], 29-85.

Llorach, R., J. C. Espin, F. A. Tomas-Barberan and F. Ferreres. 2002. Artichoke (*Cynara scolymus* L.) byproducts as a potential source of health-promoting antioxidant phenolics. J. Agric. Food Chem. 50, 3458–3464.

Lockhart, J.A.R., A. Samuel and M. P. Greaves. 1990. The evolution of weed control in British agriculture. In: Hance, R. J. and K. Holly (Eds.), Weed Control Handbook: Principles. Blackwell, Oxford. 43-74.

Louda, S. M., D. Kendall, J. Connor and D. Simberloff. 1997. Ecological effects of an insect introduced for the biological control of weeds. Science. 277, 1088-1090.

Lusk, C. H. and A. D. Pozo. 2002. Survival and growth of seedlings of 12 Chilean rainforest trees in two light environments: gas exchange and biomass distribution correlates. Austral. Ecology. 27, 173-182.

Ma, G. S., J. Q. Xue, H. D. Lue, R. H. Zhang, S. J. Tai and J. H. Ren. 2007. Effects of planting date and density on population physiological indices of summer corn (*Zea mays* L.) in central Shaanxi irrigation area. Chinese J. App. Ecol. 18 (6), 1247-1253.

Martens, S. and A. Mithoefer. 2005. Flavones and flavone synthases. Phytochem. 66, 2399-2407.

Matthes, C. and B. Honermeier. 2007. Cultivation of the artichoke as a medicinal plant under temperate climate conditions in Germany. Acta Hort. 730, 483-489.

Mauromicale, G. and A. Ierna. 2000. Characteristics of heads of seed-grown globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori] as affected by harvest period, sowing date and gibberellic acid. Agronomie. 20, 197–204.

Mauromicale, G. and A. Ierna. 1995. Effects of gibberellic acid and sowing date on harvest time and yields of seed-grown globe artichoke (*Cynara scolymus* L.). Agronomie, 15, 527-538.

Maxwell, K. and G. N. Johnson. 2000. Chlorophyll Fluorescence- a practical guide. J. Exp. Bot. 51 (345), 659-668

Methy, M., A. Olioso and L. Trabaud. 1994. Chlorophyll fluorescence as a tool for management of plant resources. Remote Sens. Environ. 47, 2-9.

Miyazawa, Y. and H. Yahata. 2006. Is the parameter electron transport rate useful as a predictor of photosynthetic carbon assimilation rate? Bull. Inst. Trop. Agr., Kyushu Univ. 29, 39-53.

Moglia, A., S. Lanteri, C. Comino, A. Acquadro, R. D. Vos and J. Beekwlider. 2008. Stress induced biosynthesis of dicaffeoylquinic acids in globe artichoke. J. Agric. Food Chem. 56, 8641-8649.

Mossi, A. J. and S. Echeverrigaray. 1999. Identification and characterization of antimicrobial components in leaf extracts of globe artichoke (*Cynara scoly*mus L.). Acta Hort. 501, 111- 114.

Owens, T. G. 1991. Energy transformation and fluorescence in photosynthesis. In: S. Demers, Editor, *Particle Analysis in Oceanography NATO ASI Series* Vol. G27, Springer-Verlag, Heidelberg, Germany. 101–137.

Palta, J. P. 1992. Mechanisms for obtaining freezing stress resistance in herbaceous plants. In Plant Breeding in the 1990s: Proceedings of a Symposium held at North Carolina State University. 219–250.

Papageorgiou, G. 1975. In: Govindjee, editor. Bioenergetics of photosynthesis. New York: Academic Press, Inc. 319–371.

Paynter, Q. E., S. V. Flower, A. H. Gourlay, M. L. Haines, H. m. Harmin, S. R. Hona, P. G. Peterson, L. A. Smith, J. R. A. Wilson-Davey, C. J. Winks and T. M. Withers. 2004. Saferty in New Zealand weed biocontrol: a nationwide survey for impacts on non-target plants. New Zealand Plant Protection. 57, 102-107.

Pemberton, R. W. 1995. *Cactoblastis cactorum* (Lepidoptera: Gracillariidae) in the United States: an immigrant biological control agent or an introduction of the nursery industry? Am. Entomol. 41, 230-232.

Percival, G. C. 2005. The use of chlorophyll fluorescence to identify chemical and environmental stress in leaf tissue of three oak (Quercus) species. J. Arboricult. 31 (5), 215-227.

Raccuia, S. A. and M. G. Melilli. 2006. Plant architecture and biomass partitioning as affected by plant density in *Cynara cardunculus* L. var. *sylvestris* Lam. Acta Hort. 730, 149-155.

Raccuia, S. A., A. Mainolfi, G. Mandolino and M. G. Melilli. 2004b. Genetic diversity in *Cynara cardunculus* revealed by AFLP markers: Comparison between cultivars and wild types from Sicily. Plant Breeding, 123, 280-284.

Raccuia, S. A., V. Cavallaro and M. G. Melilli. 2004a. Intraspecific variability in Cynara cardunculus L. var. sylvestris Lam. Sicilian populations: seed germination under salt and moisture stresses. J. Arid Environ. 56, 107-116.

Rapisarda, P., A. Tomaino, R. Lo Cascio, F. Bonina, A. De Pasquale and A. Saija. 1999. Antioxidant effectiveness as influenced by phenolic content of fresh orange juices, J. Agric. Food Chem. 47, 4718–4723.

Rohacek, K. and M. Bartak. 1999. Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. Photosynthetica. 37, 339–363.

Romani, A., P. Pinelli, C. Cantini, A. Cimato and D. Heimler. 2006. Characterization of Violetto di Toscana, a typical Italian landrace of artichoke (*Cynara scolymus* L.). Food Chem. 95, 221–225.

Schijlen E. G. W. M., C. H. R. de Vos, A. J. van Tunen, A. G. Bovy. 2004. Modification of flavonoid biosynthesis in crop plants. Phytochem. 65, 2631-2648.

Schilcher, H. 1971. Cynarinbestimmung in Artischockenblaettern und Artischockenzubereitungen. Scientia Pharmaceutica. 39, 151-154.

Schreiber, U., U. Schliwa and W. Bilger. 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth. Res. 10, 51–62.

Schreiber, U., W. Bilger and C. Neubauer. 1994. Chlorophyll fluorescence as a non intrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze, E. D. and M. M. Caldwell (Eds.): Ecophysiology of photosynthesis. Springer-Verlag, 49-70.

Schutz, K., D. Kummerer, R. Carle and A. Schieber. 2004. Identification and quantification of caffeoylquinic acids and flavonoids from artichoke (*Cynara scolymus* L.) heads, juice and pomace by HPLC-DAD-ESI/MS (n). J. Agric Food Chem. 52, 4090-4096.

Seabra, R., P. Valentao, F. Fereres and P. Andrade. 2002. Phenolic profiles in the definition of natural products authenticity: cardoon versus artichoke profile. In Natural Products in the New Millenium: Prospects and Industrial applications. 47, 183-194.

Sestak, Z., and P. Stiffel. 1997. Leaf age related differences in chlorophyll fluorescence. Photosynthetica. 33(3– 4), 347–369.

Slanina, J., E. Taborska and P. Musil. 1993. Determination of cynarin in decocticons of the artichoke (*Cynara cardunculus* L.) by the HPLC method. Cesk. Slov. Farm. 42, 275-278.

Stephanou, M. and Y. Manetas. 1997. The effects of seasons, exposure, enhanced UV-B radiation, and water stress on leaf epicuticular and internal UV-B absorbing capacity of *Cistus creticus*: a mediterranean field study. J. Exp. Bot. 48 (316), 1977-1985.

Tetio-Kagho, F. and F. P. Gardner. 1988. Response of maize to plant population density. I. Canopy development, light relationships and vegetative growth. Agron. J. 80, 930-935.

Tomlin C. D. S. 2000. The pesticide manual. 12th ed. Boston, Farnham, UK: The British Crop Protection Council. 1250-1250.

Valentao, P., E. Fernandes, F. Carvalho, P. B. Andrade, R. M. Seabra and M. L. Bastos. 2002. Antioxidative Properties of Cardoon (*Cynara cardunculus* L.) Infusion against superoxide radical, hydroxyl radical, and hypochlorous acid. J. Agric. Food Chem. 50, 4989-4993.

van Rensen, J. J. S. 1989. Herbicides interacting with photosystem II. In Herbicides and Plant Metabolism, ed. Dodge, A. D. Cambridge University Press, Cambridge. 21-36.

Walker, A. F., R. W. Middleton and O. Petrowicz. 2001. Artichoke leaf extract reduces symptoms of irritable bowl syndrome in a post-marketing surveillance study. Phytother. Res. 15, 58-61.

Ward, S. P. and O. Leyser. 2004. Shoot branching. Current opinion in plant biology. 7, 73-78

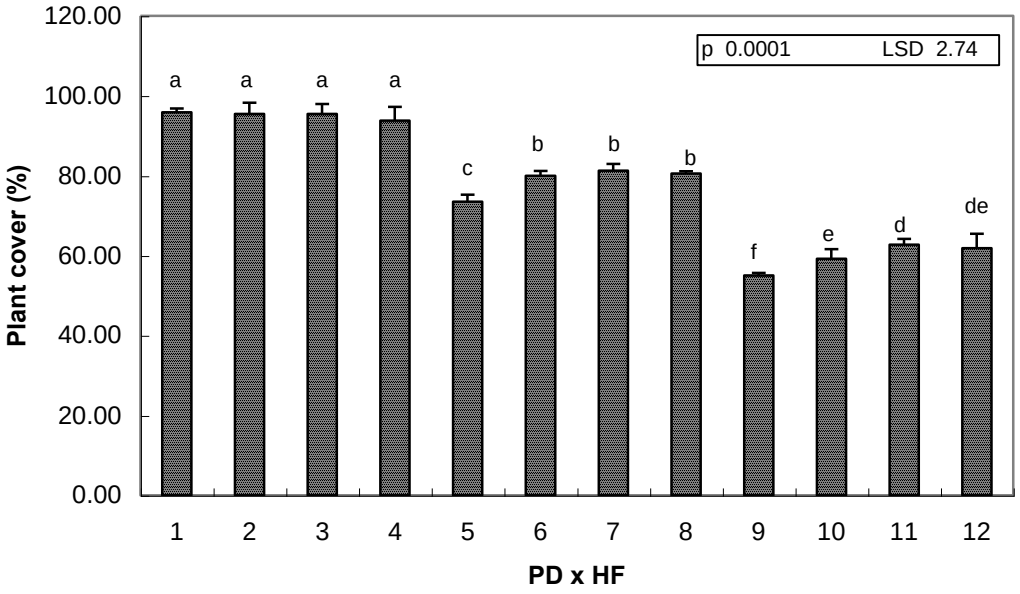
Winkel-Shirley, B. 2001. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology and biotechnology. Plant Physiol. 126, 485-493.

Wong, T. S. and P. Couture. 1986. Toxicity screening using phytoplankton. In: B.J. Dutka and G. Bitton, Editors, *Toxicity testing using microorganisms* Vol. 2, CRC Press Inc, Boca Raton, Florida. 79–100.

Yamasaki, S., Noguchi, N. and Mimaki, K. 2007. Continuous UV-B irradiation induces morphological changes and the accumulation of polyphenolic compounds on the surface of cucumber cotyledons. J. Radiat. Res. 48, 443-453.

Zhu, X., H. Zhang, and R. Lo. 2004. Phenolic compounds from the leaf extract of artichoke (*Cynara scolymus* L.) and their antimicrobial activities. J. Agric. Food Chem. 52, 7272-7278.

8. Appendices



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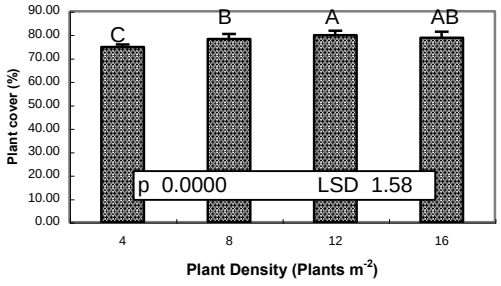
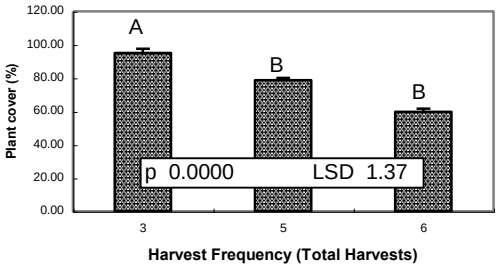
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

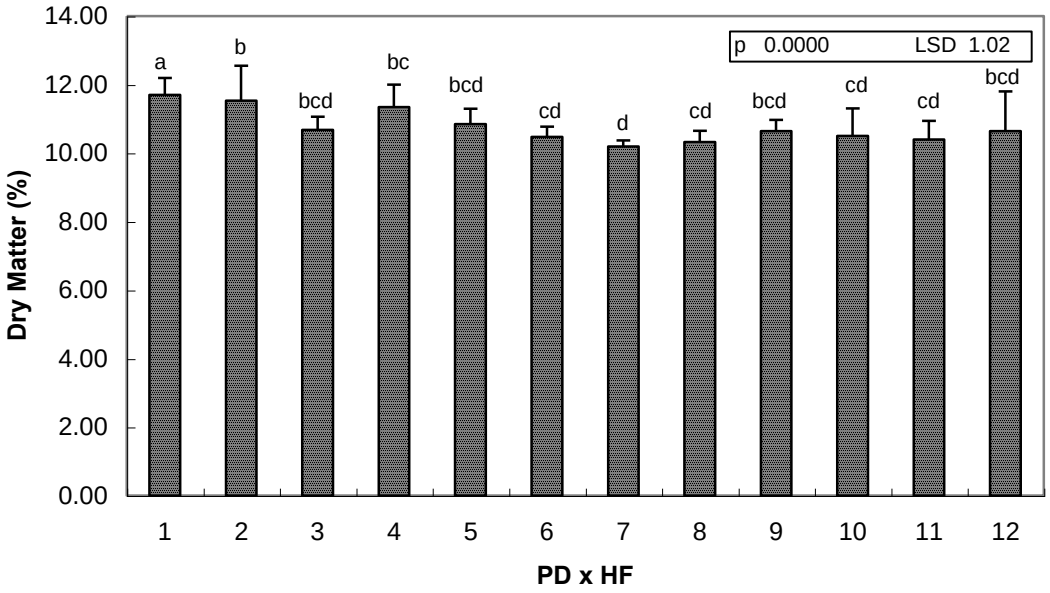
Appendix A1: Effect of plant density and harvest frequency on plant cover (%) in artichoke Gross Gerau, 2006 (T = ±SD)



Appendix A2: Plant cover (%) as affected by plant density, Gross Gerau 2006 (T = ±SD)

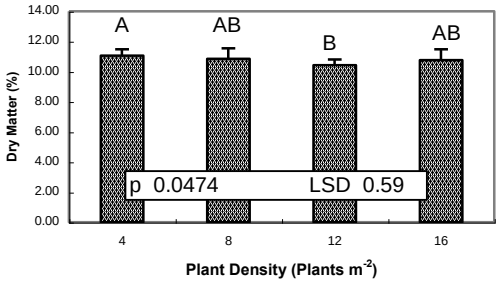


Appendix A3: Plant cover (%) as affected by harvest frequency, Gross Gerau 2006 (T = ±SD)

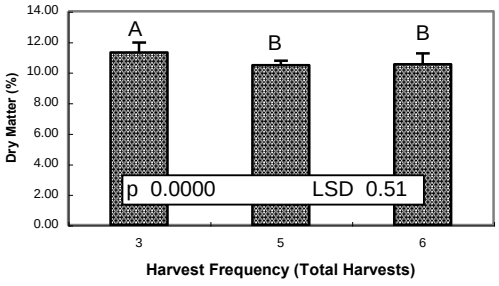


1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

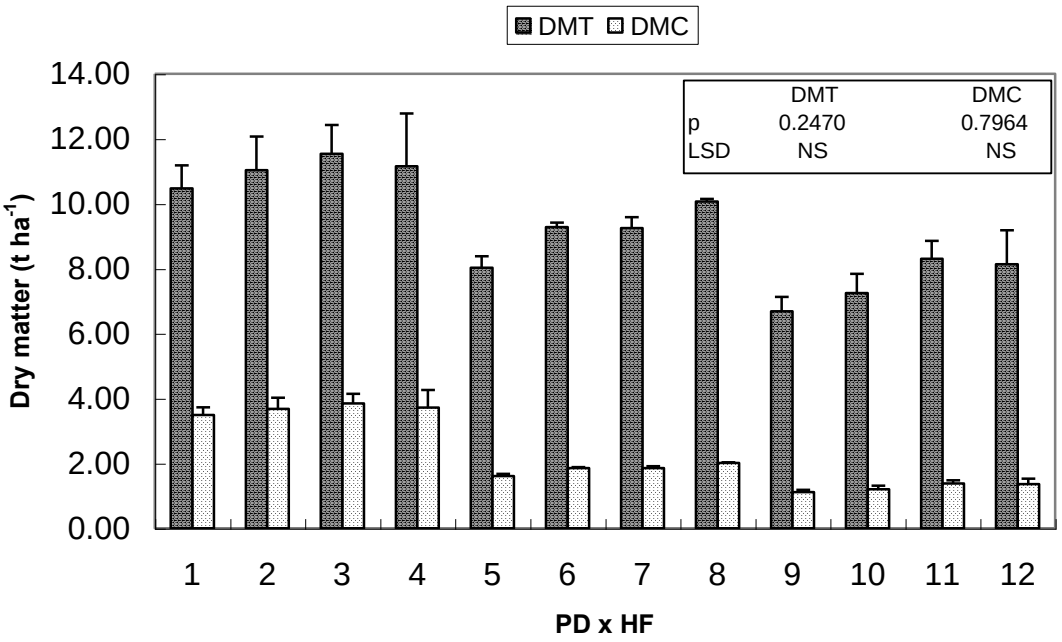
Appendix A4: Effect of plant density and harvest frequency on dry matter (%) in artichoke, Gross Gerau 2006 (T = ±SD)



Appendix A5: Dry matter (%) as affected by plant density, Gross Gerau 2006 (T = ±SD)

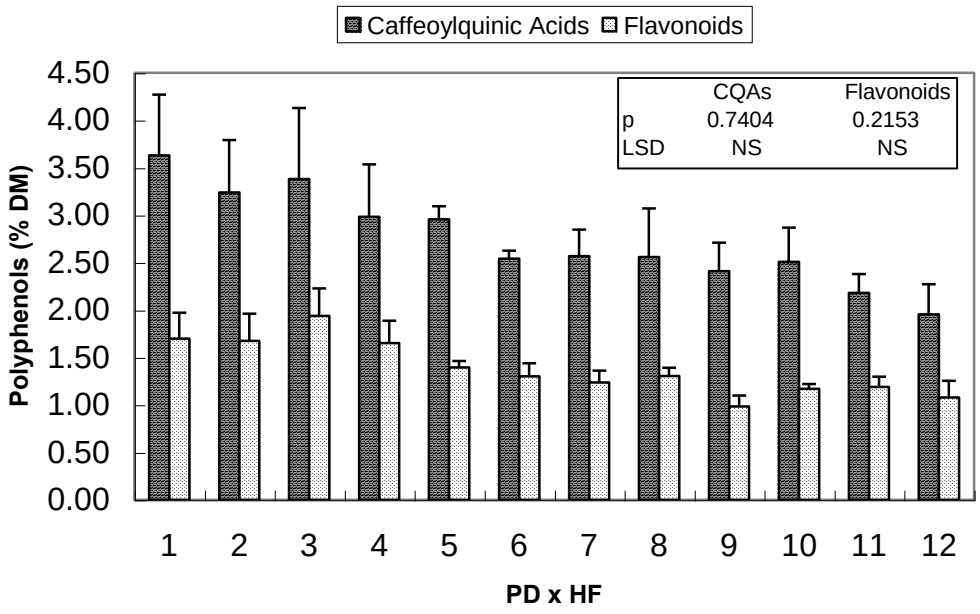


Appendix A6: Dry matter (%) as affected by harvest frequency, Gross Gerau 2006 (T = ±SD)



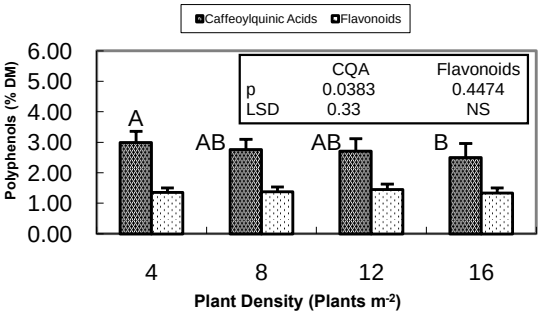
1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

Appendix A7: Effect of plant density and harvest frequency on dry matter (t ha^{-1}) in artichoke, Gross Gerau 2006 (T = ±SD)

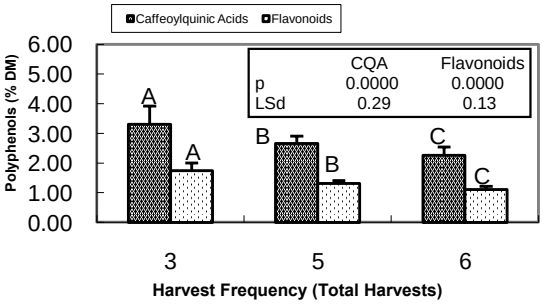


1: 4 Pl m^{-2} x 3 cuts	**2:** 8 Pl m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

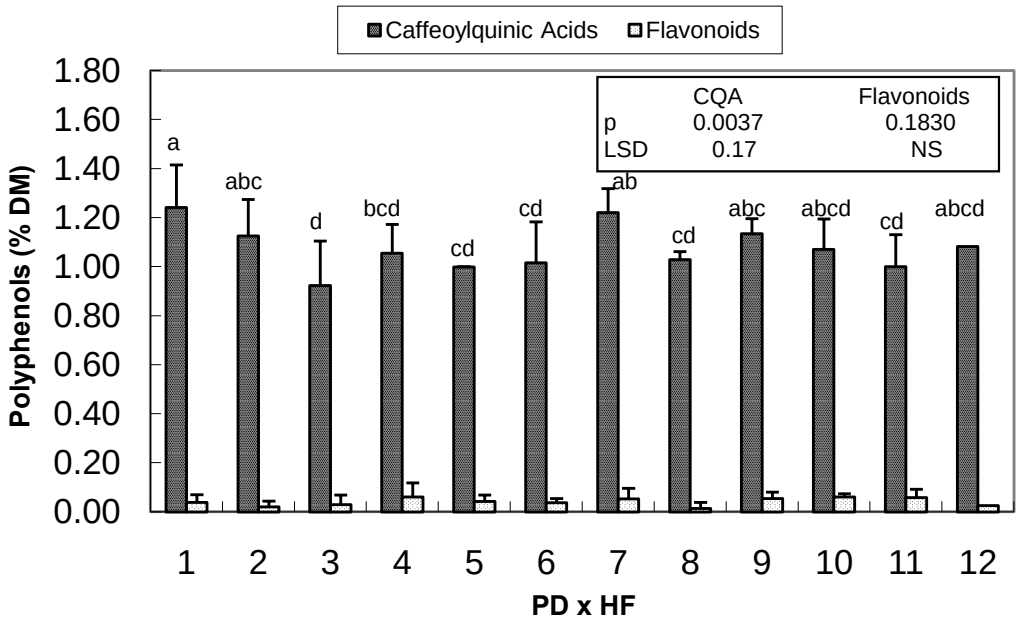
Appendix A8: Effect of plant density and harvest frequency on polyphenols (% DM) in artichoke leaf blades, Gross Gerau 2006 (T = ±SD)



Appendix A9: Polyphenols (% DM) in artichoke leaf blades as affected by plant density, Gross Gerau 2006 (T = ±SD)

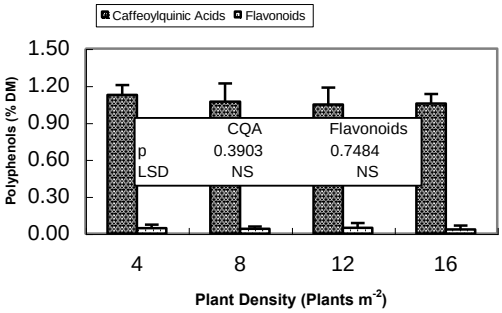


Appendix A10: Polyphenols (% DM) in artichoke leaf blades as affected by harvest frequency, Gross Gerau 2006 (T = ±SD)

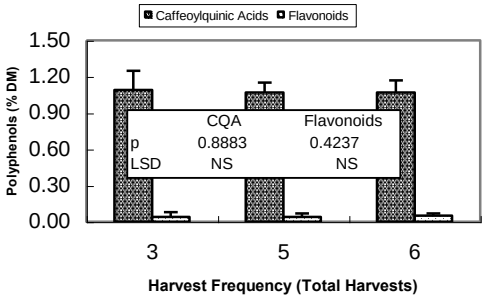


1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

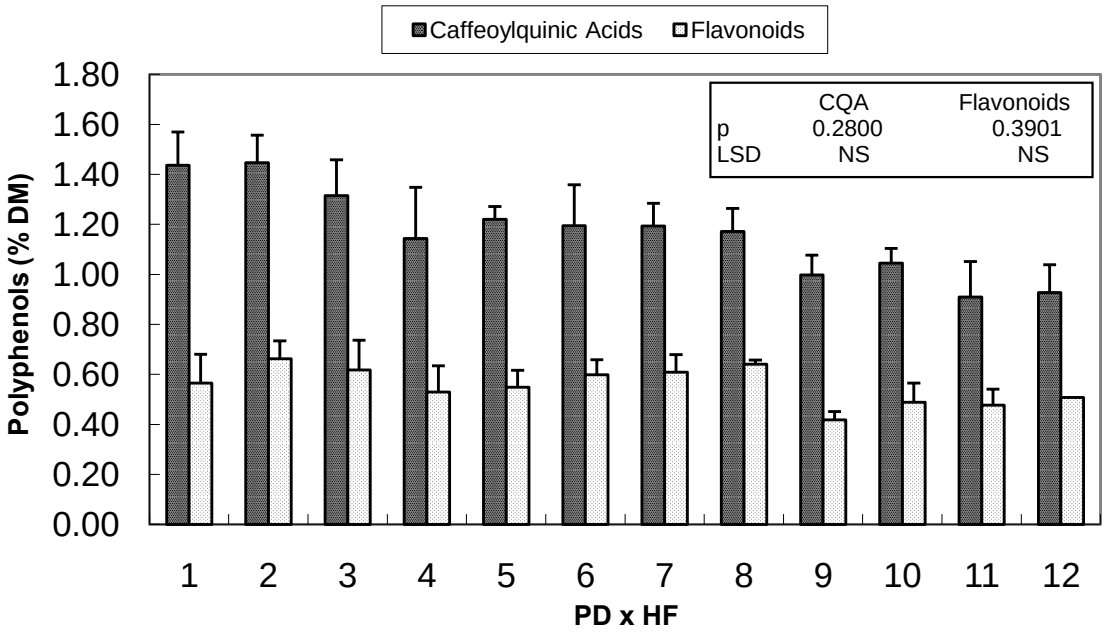
Appendix A11: Effect of plant density and harvest frequency on polyphenols (% DM) in artichoke leaf veins, Gross Gerau 2006 (T = ±SD)



Appendix A12: Polyphenols (% DM) in artichoke leaf veins as affected by plant density, Gross Gerau 2006 (T = ±SD)

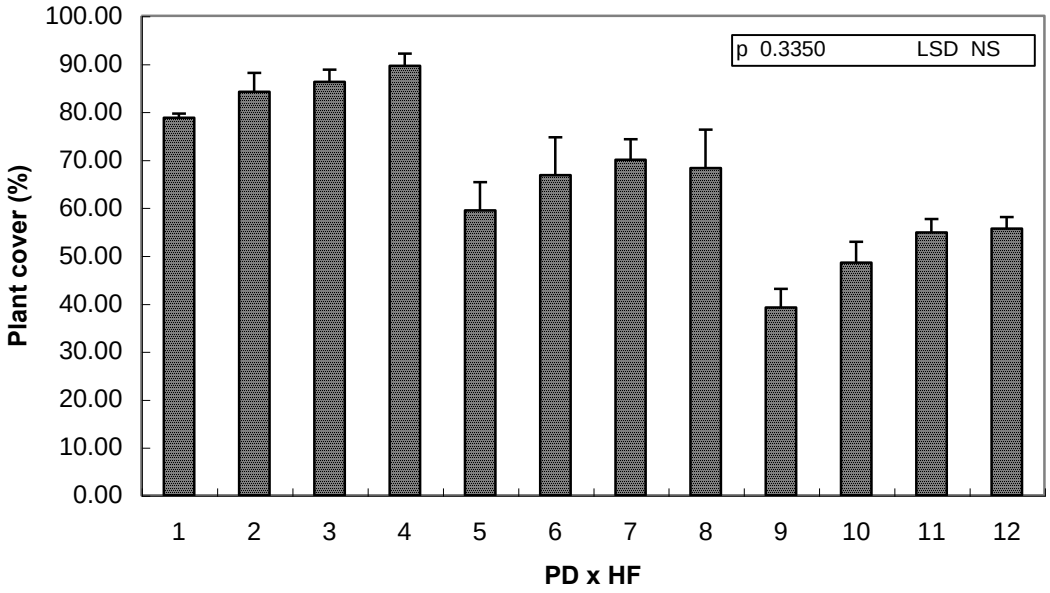


Appendix A13: Polyphenols (% DM) in artichoke leaf veins as affected by harvest frequency, Gross Gerau 2006 (T = ±SD)



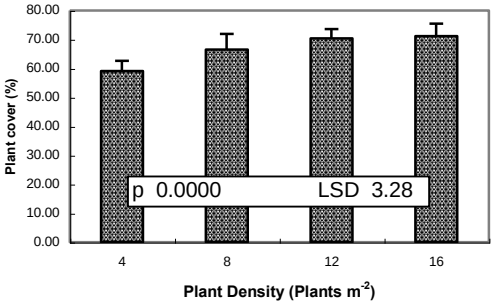
1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

Appendix A14: Effect of plant density and harvest frequency on polyphenols (% DM) in artichoke leaves (whole leaves), Gross Gerau 2006 (T = ±SD)

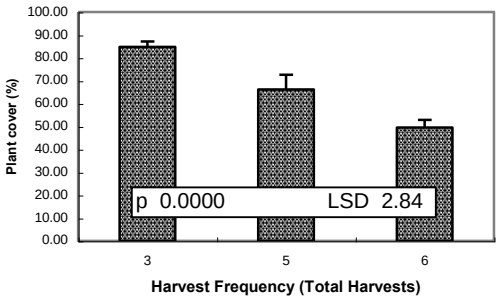


1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

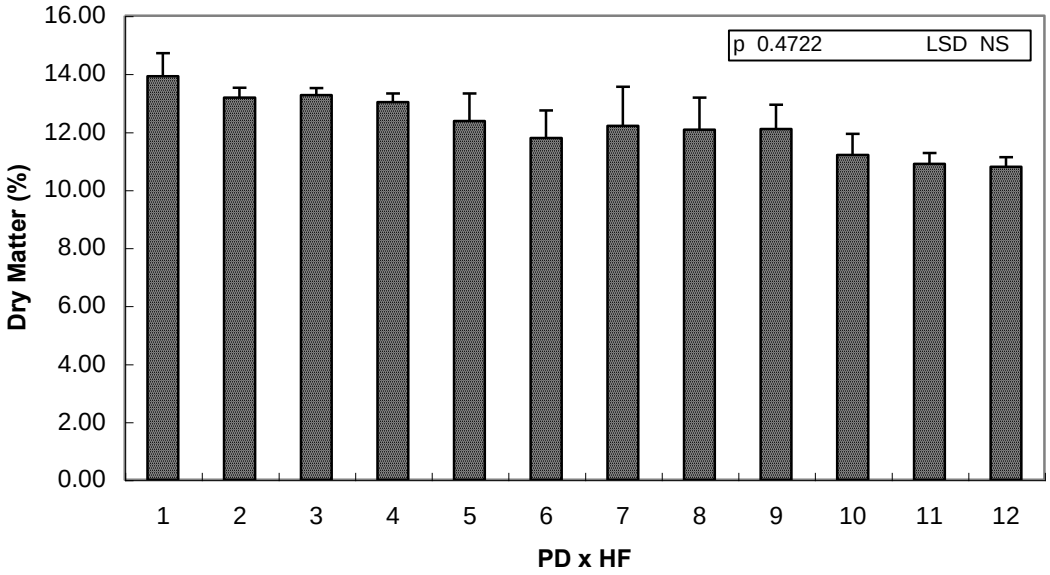
Appendix A15: Effect of plant density and harvest frequency on plant cover (%) in artichoke, Gross Gerau 2007 (T = ±SD)



Appendix A16: Plant cover (%) as affected by plant density, Gross Gerau 2007 (T = ±SD)

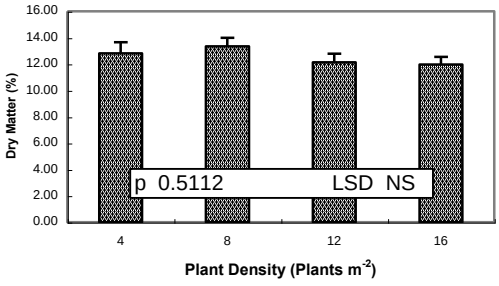


Appendix A17: Plant cover (%) as affected by harvest frequency, Gross Gerau 2007 (T = ±SD)

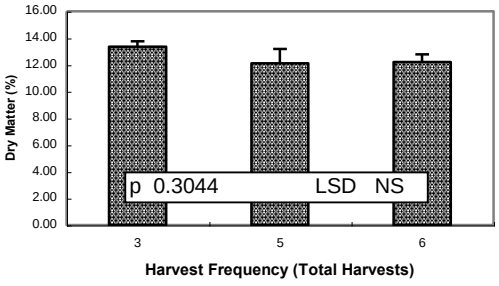


1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

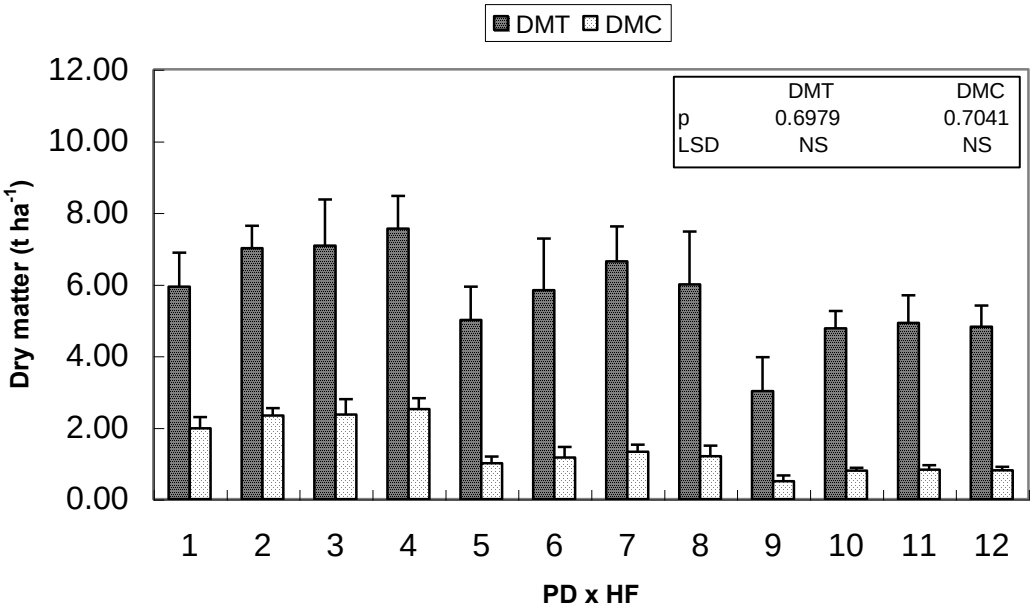
Appendix A18: Effect of plant density and harvest frequency on dry matter (%) in artichoke, Gross Gerau 2007 (T = ±SD)



Appendix A19: Dry matter (%) as affected by plant density, Gross Gerau 2007 (T = ±SD)

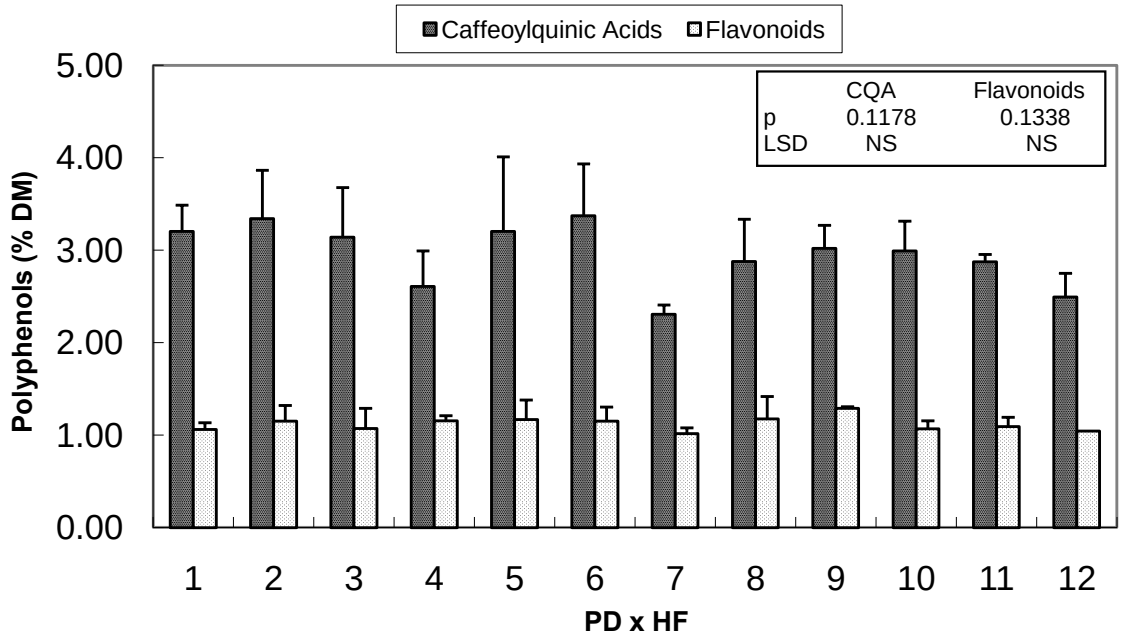


Appendix A20: Dry matter (%) as affected by harvest frequency, Gross Gerau 2007 (T = ±SD)



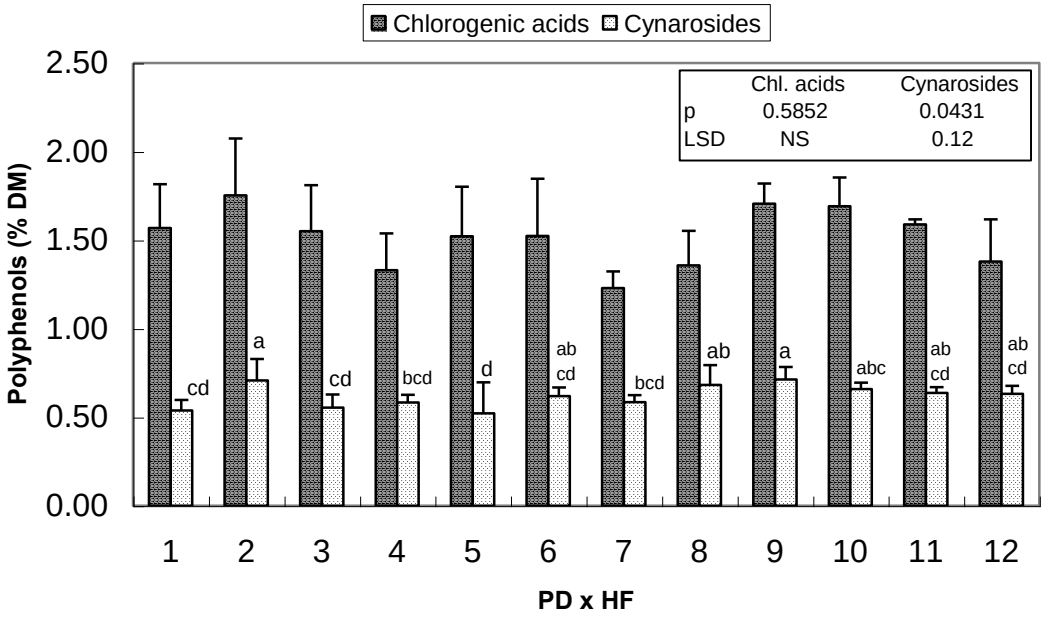
1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

Appendix A21: Effect of plant density and harvest frequency on dry matter (t ha^{-1}) in artichoke, Gross Gerau 2007 (T = ±SD)



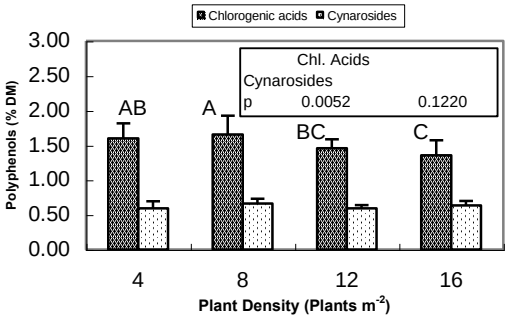
1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

Appendix A22: Effect of plant density and harvest frequency on caffeoylquinic acids and flavonoids (% DM) in artichoke leaves, Gross Gerau 2007 (T = ±SD)

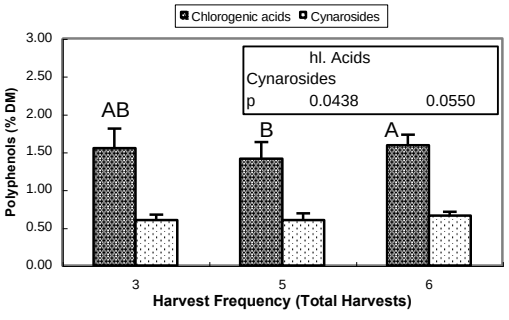


1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

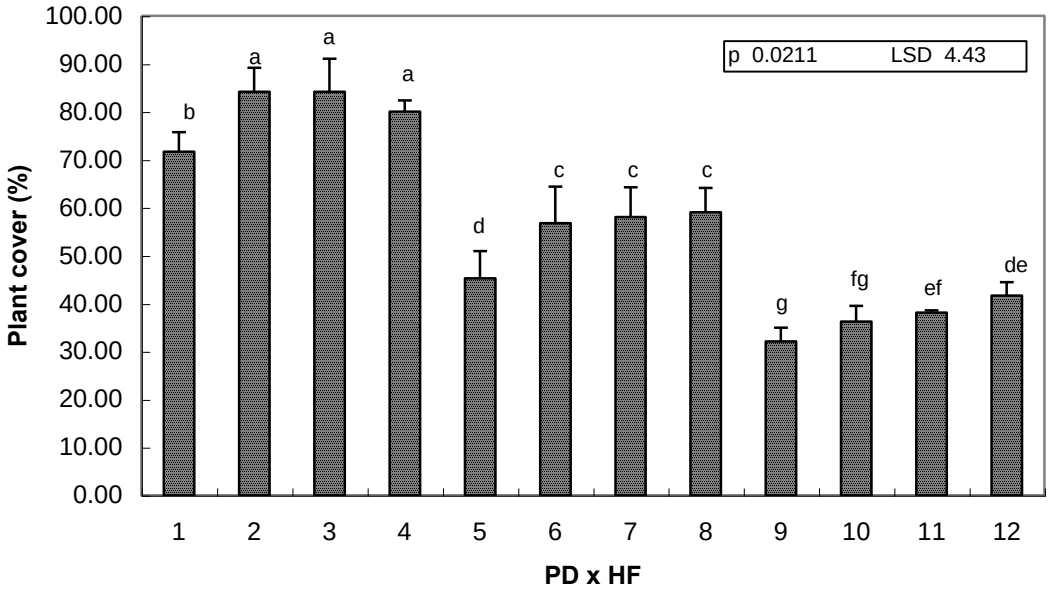
Appendix A23: Effect of plant density and harvest frequency on chlorogenic acids and cynarosides (% DM) in artichoke leaves, Gross Gerau 2007 (T = ±SD)



Appendix A24: Chlorogenic acids and cynarosides (% DM) in artichoke leaves as affected by plant density, Gross Gerau 2007 (T = ±SD)

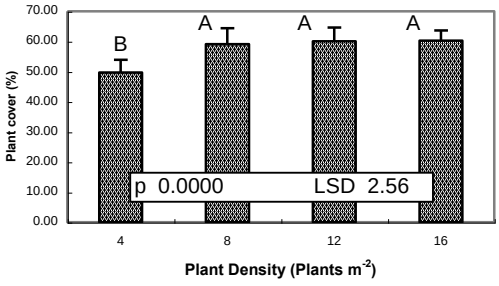


Appendix A25: Chlorogenic acids and cynarosides (% DM) in artichoke leaves as affected by harvest frequency, Gross Gerau 2007 (T = ±SD)

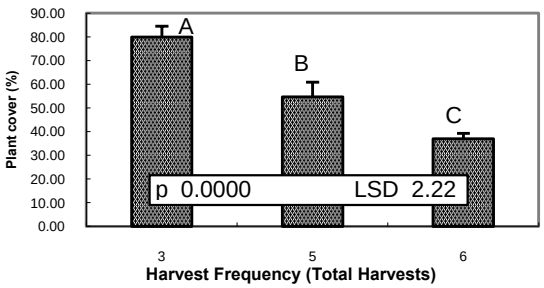


1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

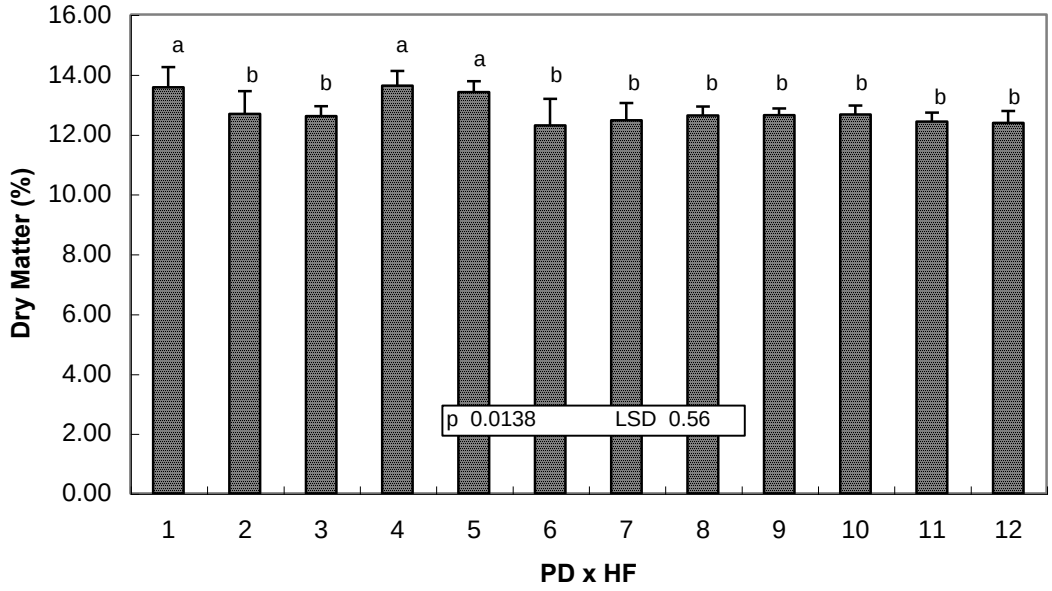
Appendix A26: Effect of plant density and harvest frequency on plant cover (%) in artichoke, Gross Gerau 2008 (T = ±SD)



Appendix A27: Plant cover (%) as affected by plant density, Gross Gerau 2008 (T = ±SD)

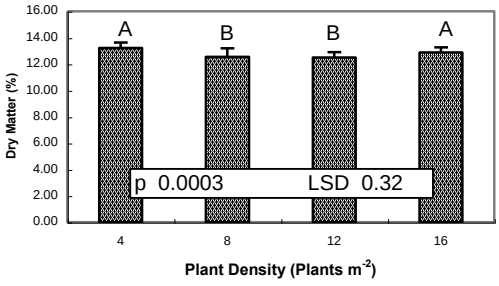


Appendix A28: Plant cover (%) as affected by harvest frequency, Gross Gerau 2008 (T = ±SD)

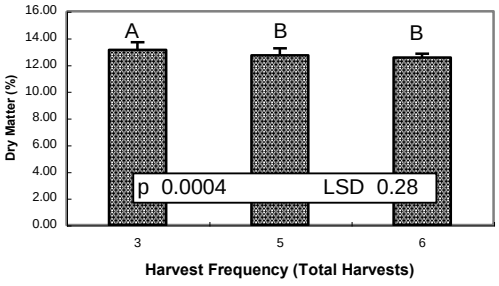


1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

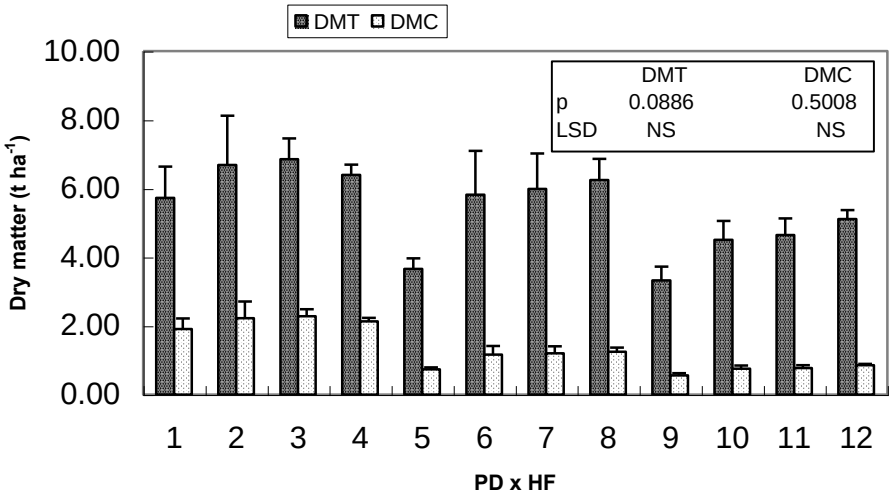
Appendix A29: Effect of plant density and harvest frequency on dry matter (%) in artichoke, Gross Gerau 2008 (T = ±SD)



Appendix A30: Dry matter (%) as affected by plant density, Gross Gerau 2008 (T = ±SD)

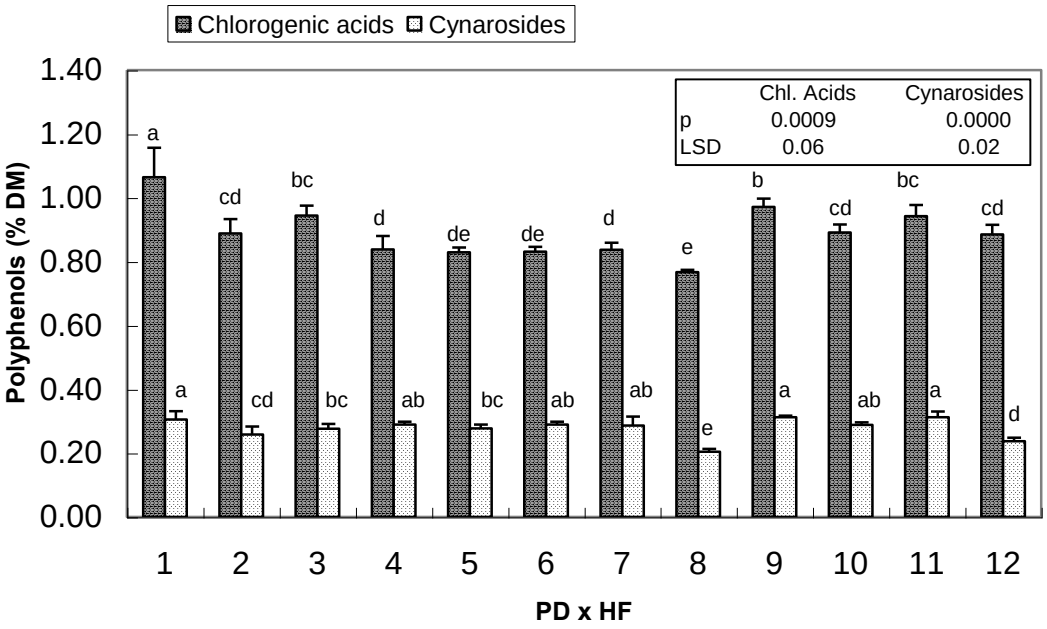


Appendix A31: Dry matter (%) as affected by harvest frequency, Gross Gerau 2008 (T = ±SD)



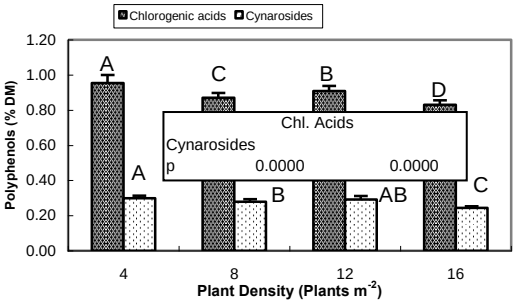
1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

Appendix A32: Effect of plant density and harvest frequency on dry matter (t ha^{-1}) in artichoke, Gross Gerau 2008 (T = ±SD)

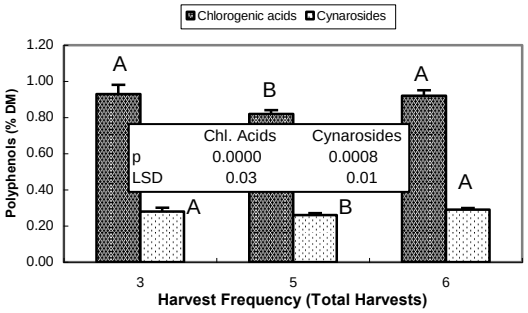


1: 4 Pl. m^{-2} x 3 cuts	**2:** 8 Pl. m^{-2} x 3 cuts	**3:** 12 Pl m^{-2} x 3 cuts	**4:** 16 Pl m^{-2} x 3 cuts
5: 4 Pl m^{-2} x 5 cuts	**6:** 8 Pl m^{-2} x 5 cuts	**7:** 12 Pl m^{-2} x 5 cuts	**8:** 16 Pl m^{-2} x 5 cuts
9: 4 Pl m^{-2} x 6 cuts	**10:** 8 Pl m^{-2} x 6 cuts	**11:** 12 Pl m^{-2} x 6 cuts	**12:** 16 Pl m^{-2} x 6 cuts

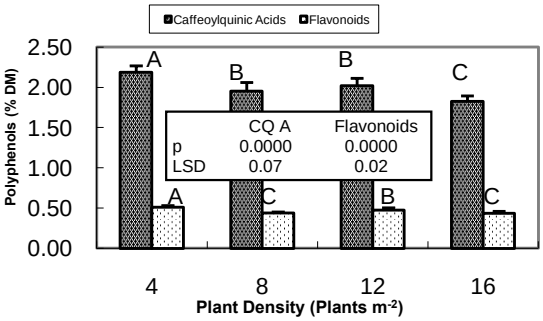
Appendix A33: Effect of plant density and harvest frequency on chlorogenic acids and cynarosides (% DM) in artichoke leaves, Gross Gerau 2008 (T = ±SD)



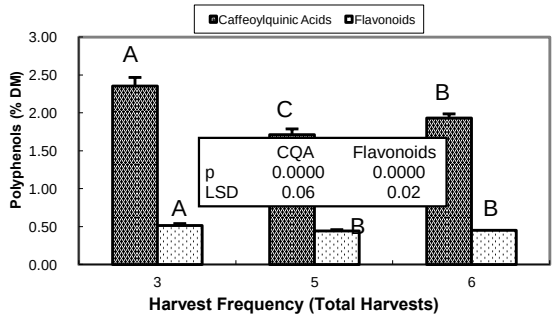
Appendix A34: Chlorogenic acids and cynarosides (% DM) in artichoke leaves as affected by plant density, Gross Gerau 2008 (T = ±SD)



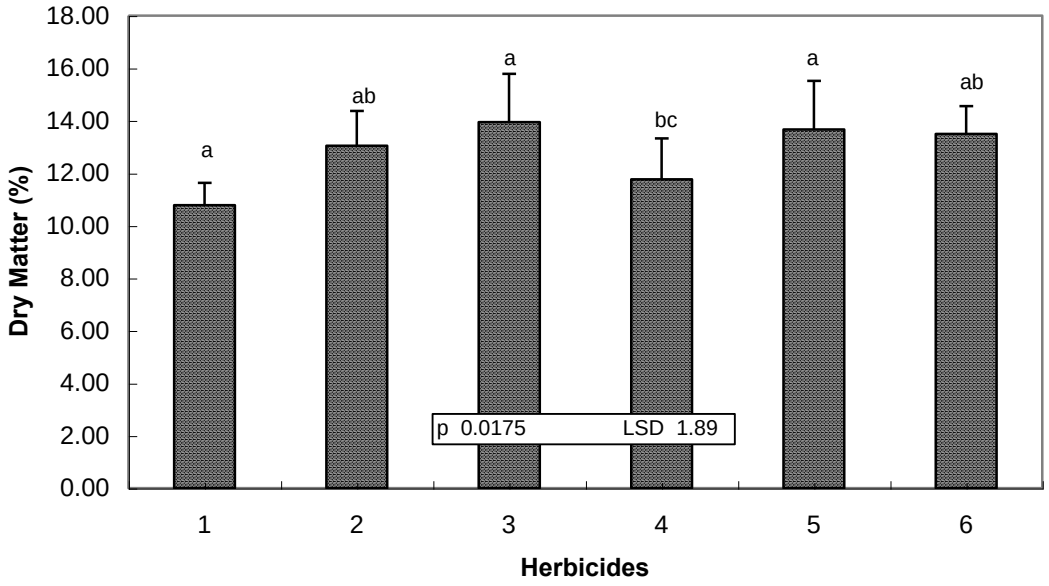
Appendix A35: Chlorogenic acids and cynarosides (% DM) in artichoke leaves as affected by harvest frequency, Gross Gerau 2008 (T = ±SD)



Appendix A36: CQA and flavonoids (% DM) in artichoke leaves as affected by plant density, Gross Gerau 2008 (T = ±SD)

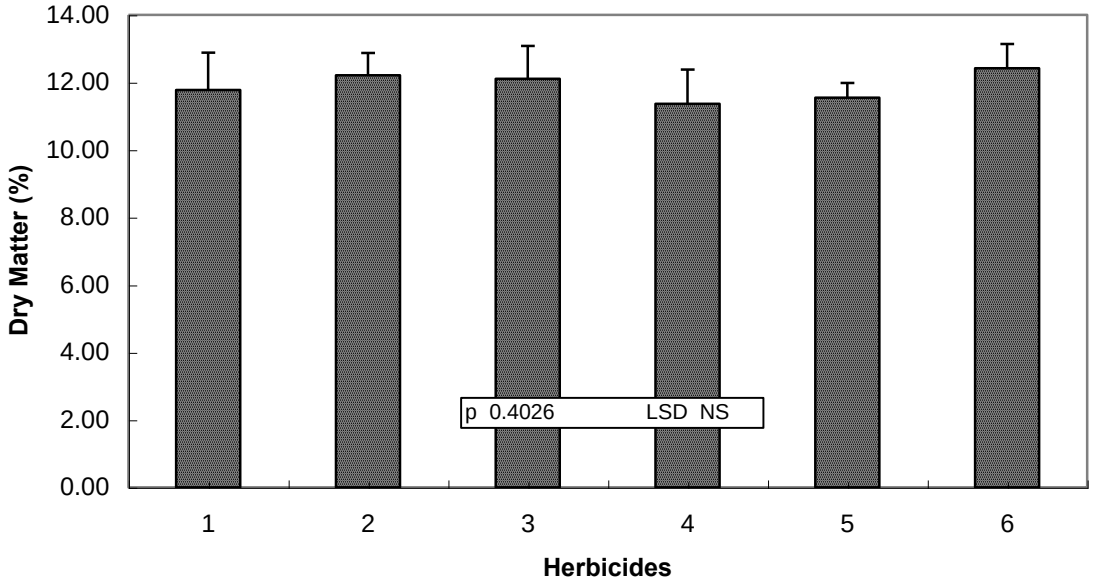


Appendix A37: CQA and flavonoids (% DM) in artichoke leaves as affected by harvest frequency, Gross Gerau 2008 (T = ±SD)



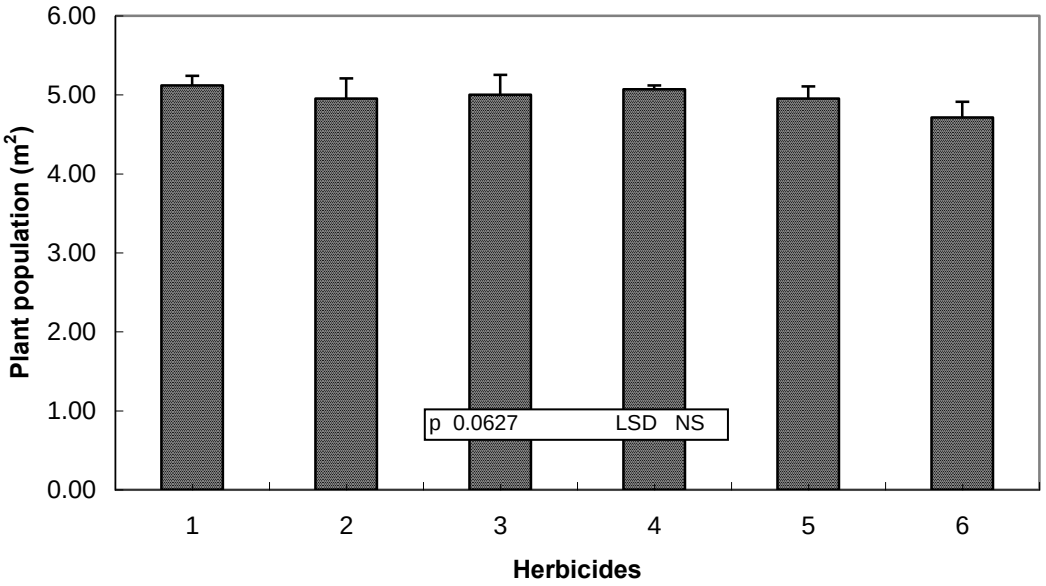
1: Control	**2:** Haloxyfop	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P	**6:** Prosulfocarb

Appendix A38: Effect of herbicides on dry matter (%) of artichoke, Giessen 1st growth phase, 2006 (T = ±SD)



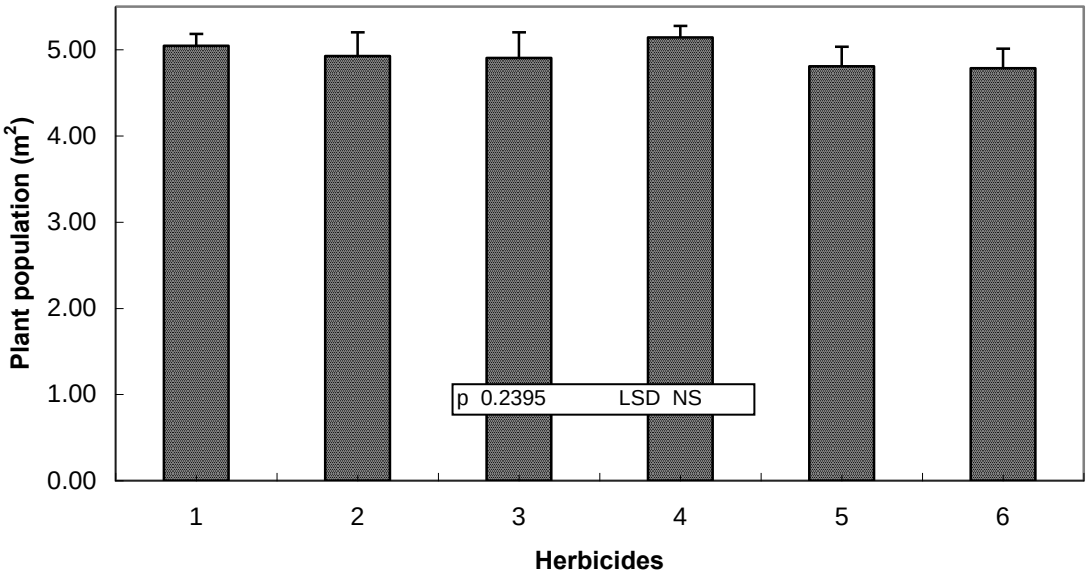
1: Control	**2:** Haloxyfop	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P	**6:** Prosulfocarb

Appendix A39: Effect of herbicides on dry matter (%) of artichoke, Giessen 2nd growth phase, 2006 (T = ±SD)



1: Control	**2:** Haloxyfop	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P	**6:** Prosulfocarb

Appendix A40: Effect of herbicides on plant population (m^2) of artichoke, Giessen 1st growth phase, 2006 (T = ±SD)



1: Control	**2:** Haloxyfop	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P	**6:** Prosulfocarb

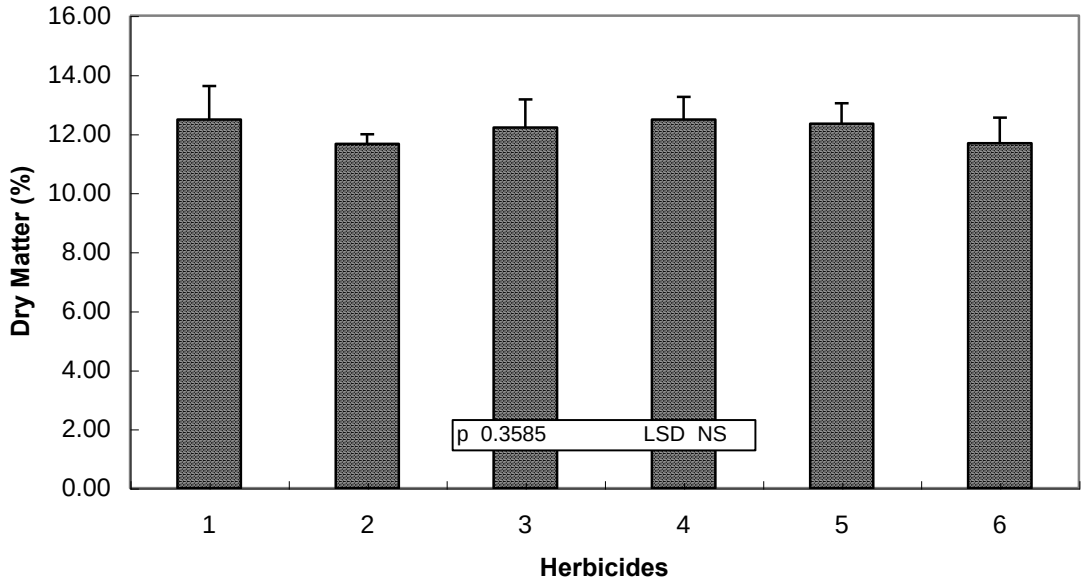
Appendix A41: Effect of herbicides on plant population (m^2) of artichoke, Giessen 2nd growth phase, 2006 (T = ±SD)

1: Control	**2:** Haloxyfop	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P	**6:** Prosulfocarb

Appendix A42: Effect of herbicides on plant cover (%) of artichoke, Giessen 1st growth phase, 2006 (T = ±SD)

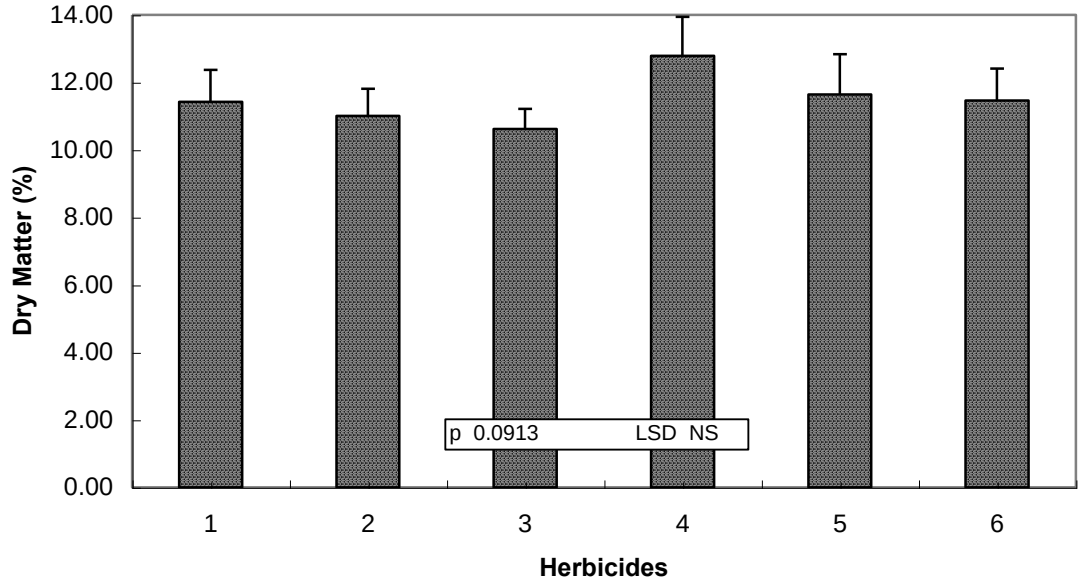
1: Control	**2:** Haloxyfop	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P	**6:** Prosulfocarb

Appendix A43: Effect of herbicides on plant cover (%) of artichoke, Giessen 2nd growth phase, 2006 (T = ±SD)



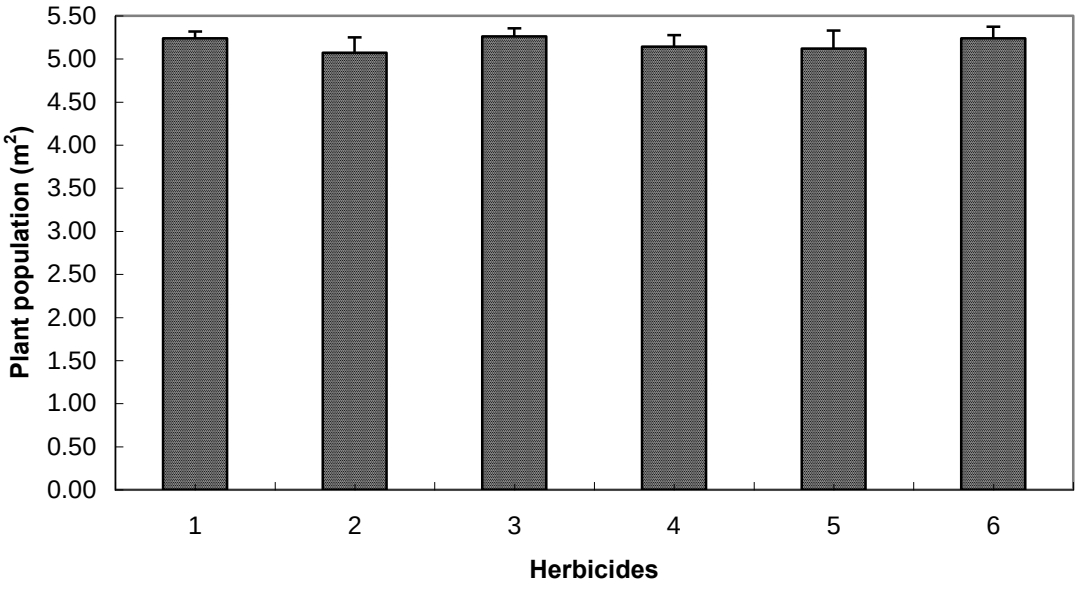
1: Control	**2:** Haloxyfop	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P	**6:** Prosulfocarb

Appendix A44: Effect of herbicides on dry matter (%) of artichoke, Giessen 1st growth phase, 2007 (T = ±SD)



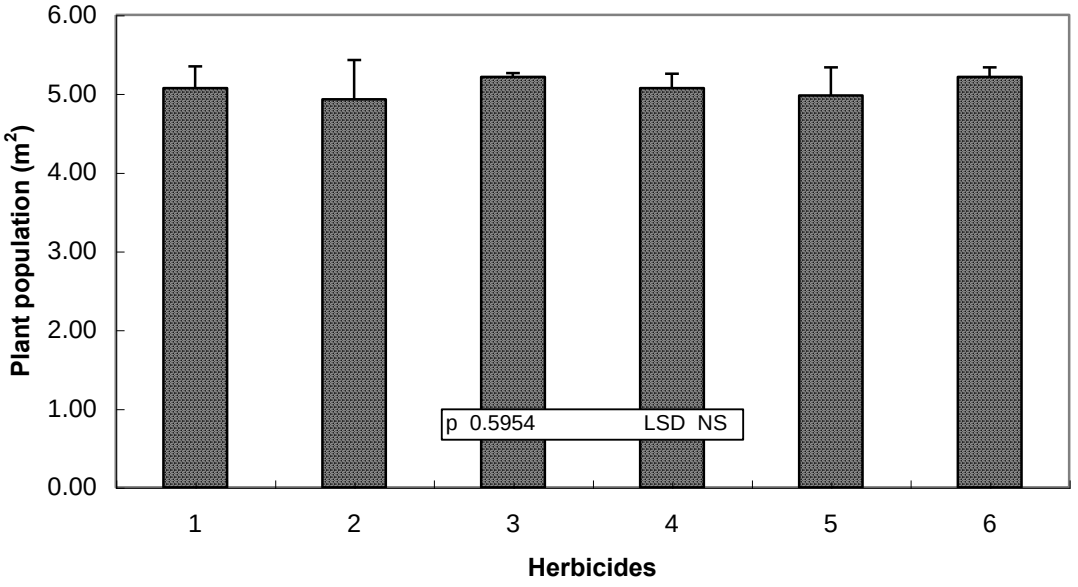
1: Control	**2:** Haloxyfop	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P	**6:** Prosulfocarb

Appendix A45: Effect of herbicides on dry matter (%) of artichoke Giessen 2nd growth phase, 2007 (T = ±SD)



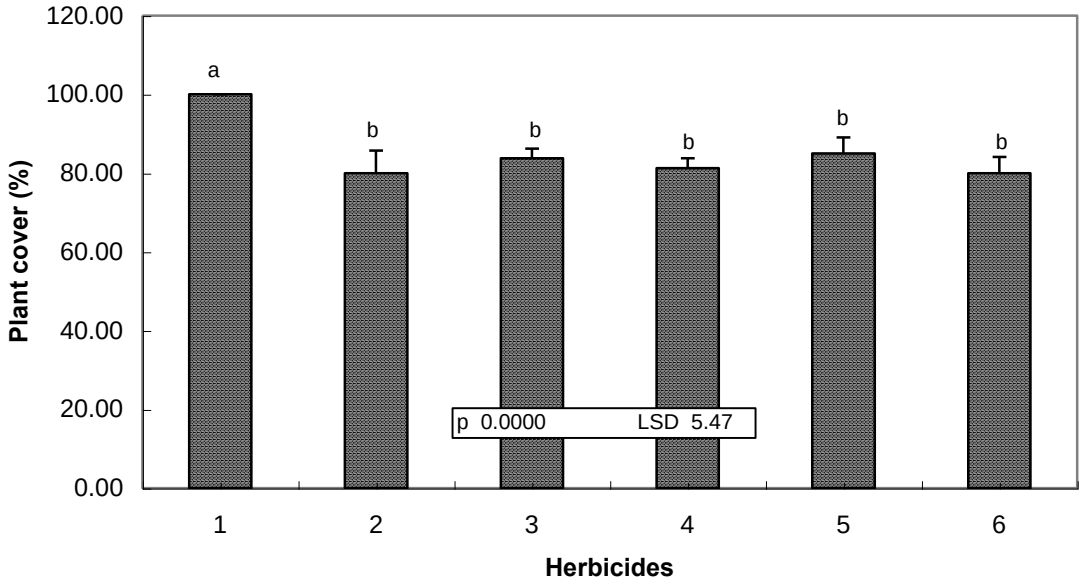
1: Control	**2:** Haloxyfop	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P	**6:** Prosulfocarb

Appendix A46: Effect of herbicides on plant population (m^2) of artichoke, Giessen 1st growth phase, 2007 (T = ±SD)



1: Control	**2:** Haloxyfop	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P	**6:** Prosulfocarb

Appendix A47: Effect of herbicides on plant population (m^2) of artichoke, Giessen 2nd growth phase, 2007 (T = ±SD)

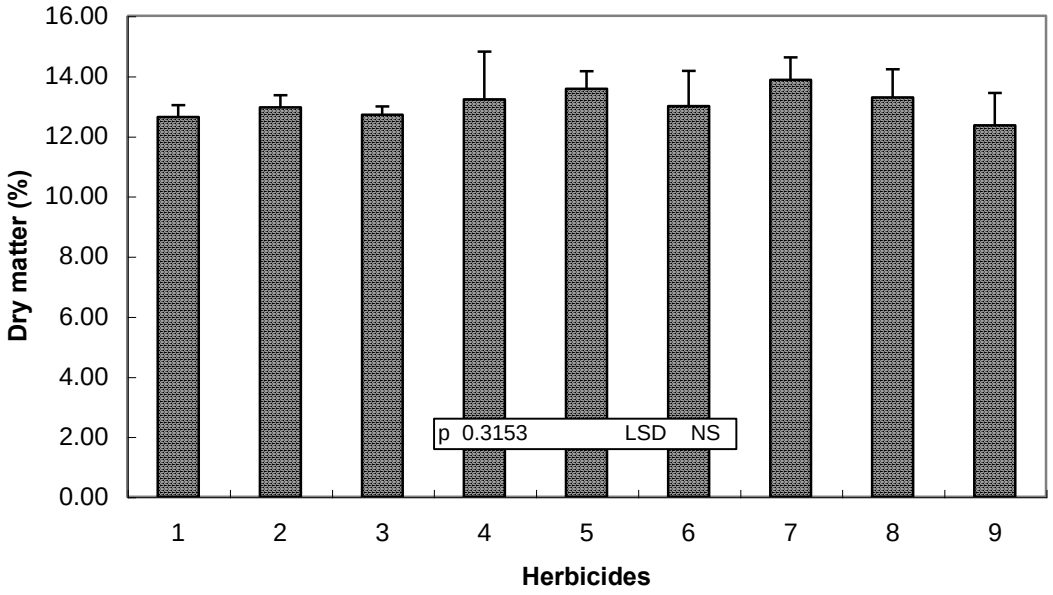


1: Control	**2:** Haloxyfop	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P	**6:** Prosulfocarb

Appendix A48: Effect of herbicides on plant cover (%) of artichoke, Giessen 1st growth phase, 2007 (T = ±SD)

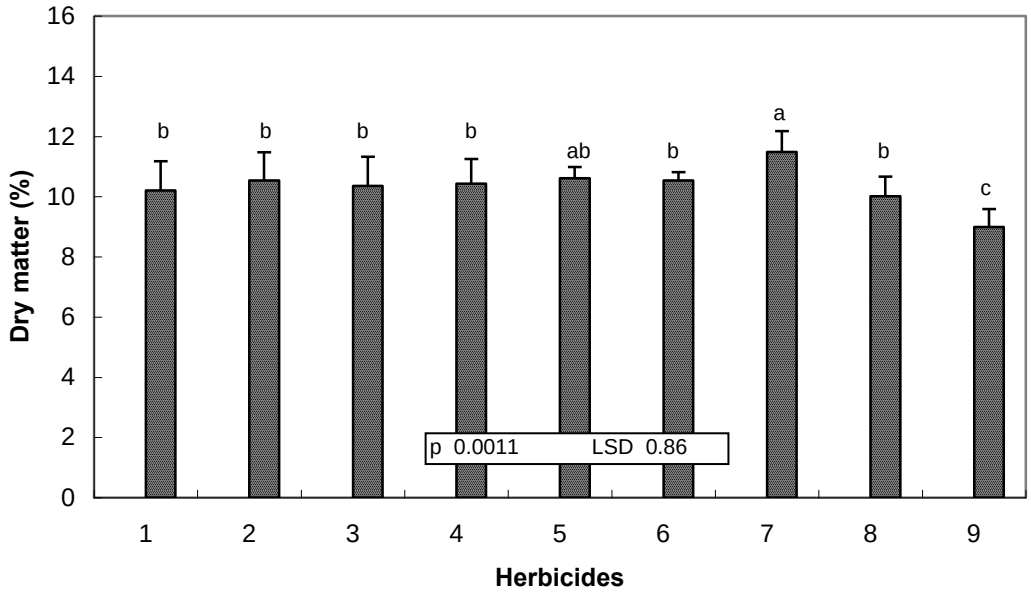
1: Control	**2:** Haloxyfop	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P	**6:** Prosulfocarb

Appendix A49: Effect of herbicides on plant cover (%) of artichoke, Giessen 2nd growth phase, 2007 (T = ±SD)



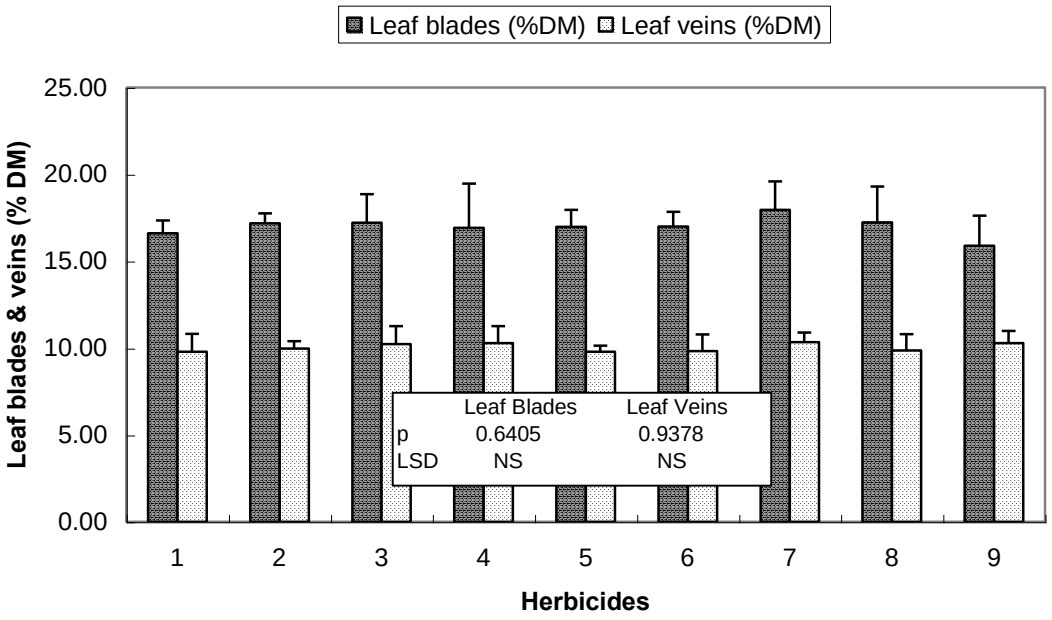
1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Appendix A50: Effect of herbicides on dry matter (%) of artichoke, Giessen 1st growth phase, 2008 (T = ±SD)



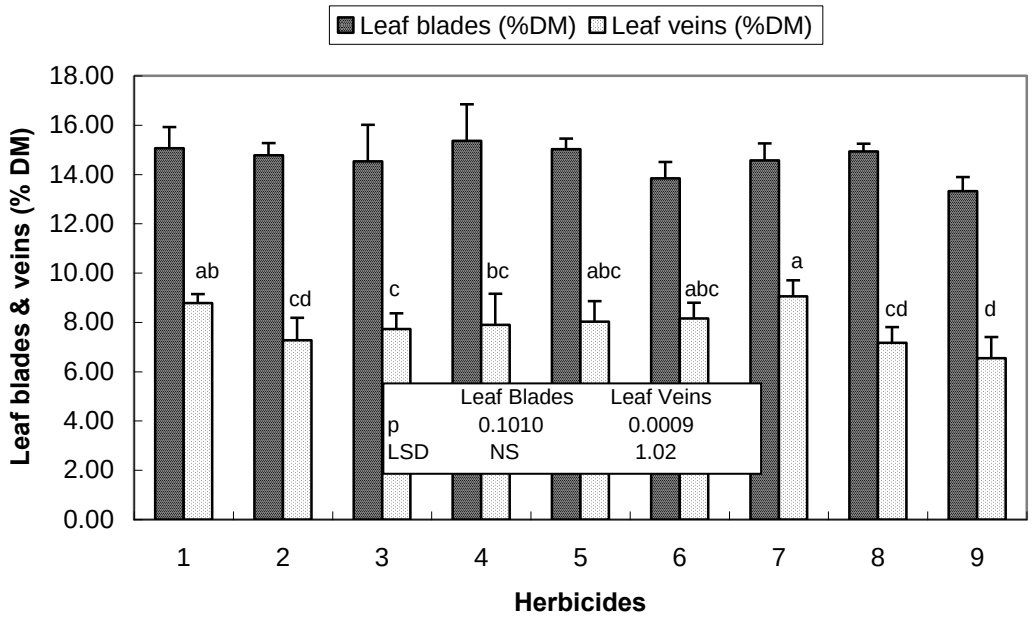
1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Appendix A51: Effect of herbicides on dry matter (%) of artichoke, Giessen 2nd growth phase, 2008 (T = ±SD)



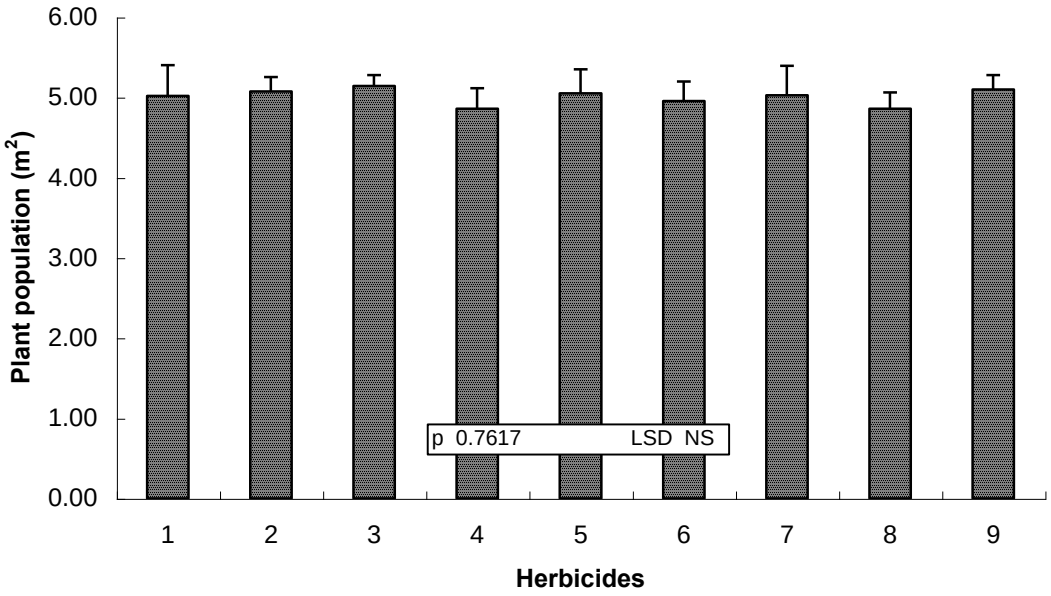
1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Appendix A52: Effect of herbicides on leaf blades and veins (% DM) of artichoke, Giessen 1st growth phase, 2008 (T = ±SD)



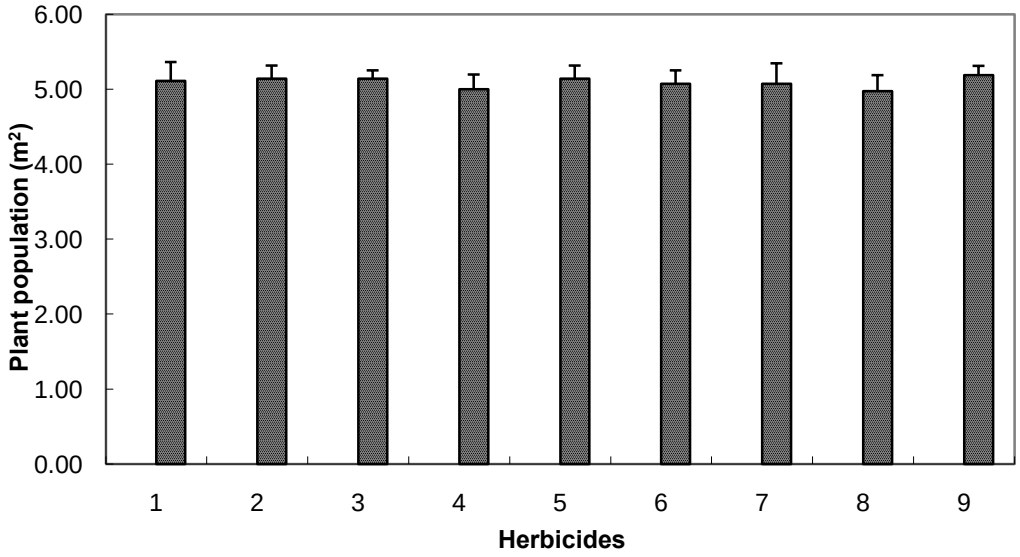
1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Appendix A53: Effect of herbicides on leaf blades and veins (% DM) of artichoke, Giessen 2nd growth phase, 2008 (T = ±SD)



1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Appendix A54: Effect of herbicides on plant population (m^2) of artichoke, Giessen 1st growth phase, 2008 (T = ±SD)

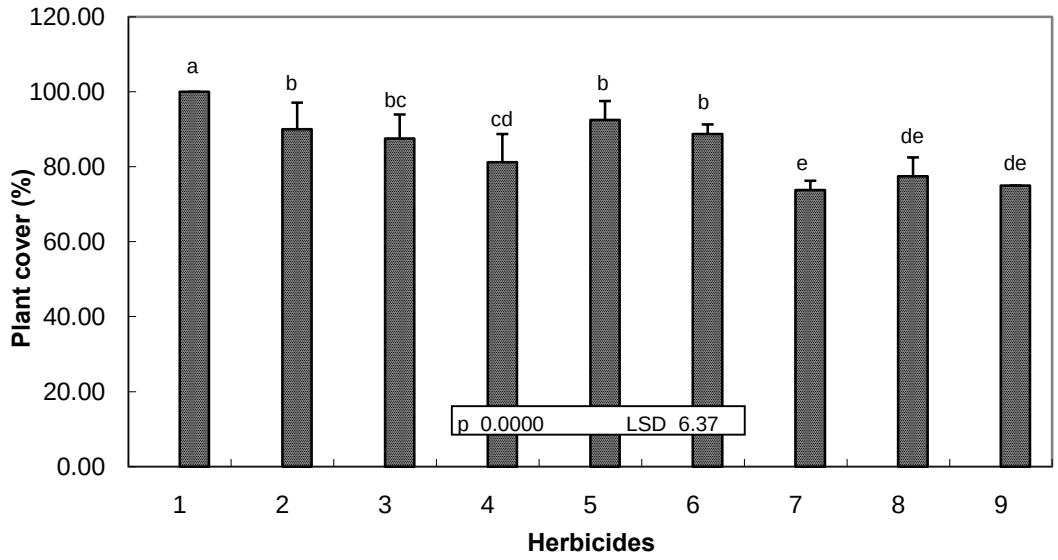


1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Appendix A55: Effect of herbicides on plant population (m^2) of artichoke, Giessen 2nd growth phase, 2008 (T = ±SD)

1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Appendix A56: Effect of herbicides on plant cover (%) of artichoke, Giessen 1st growth phase, 2008 (T = ±SD)



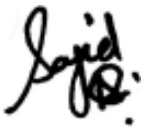
1: Control	**2:** Carfentrazone	**3:** Phenmedipham	**4:** Pyridate	**5:** Quizalofop-P
6: Prosulfocarb	**7:** Rimsulfuron	**8:** Aclonifen	**9:** Clomazone	

Appendix A57: Effect of herbicides on plant cover (%) of artichoke, Giessen 2nd growth phase, 2008 (T = ±SD)

Declaration / Erklärung

I declare: this dissertation submitted is a work of my own, written without any illegitimate help by any third party and only with materials indicated in the dissertation. I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me. At any time during the investigations carried out by me and described in the dissertation, I followed the principles of good scientific practice as defined in the "Statutes of the Justus Liebig University Giessen for the Safeguarding of Good Scientific Practice".

„Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis" niedergelegt sind, eingehalten."



Giessen, April 6, 2011 **(Sajid Ali)**

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I am finally to admit that errors that remain are mine

Sajid Ali

Der Lebenslauf wurde aus der elektronischen Version der Arbeit entfernt.

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