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Phytotoxicity and Residual Effect of Some Herbicides on three Egyptian Rice Cultivars (*Oryza sativa* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The current study was carried out at the experimental farm of Rice Research Department, Sakha Agricultural Research Station and Central Agricultural Pesticide Laboratory, ARC, Egypt during the summer season of 2021 and 2022 to study the sensitivity of three Egyptian rice cultivars against four common herbicides and its effect on physiological and agronomic characteristics of rice as well as herbicide residues in rice seeds. The field study was laid out in split plot design with three replications. Three rice cultivars (Giza 177, Giza 179 and Sakha 108) were randomly distributed in

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main plots, while weed control treatments included four herbicides at recommended doses (penoxsulam 2.5% OD, bispyribac-sodium 2% SL, cyhalofop-butyl 10% EC and fenoxaprop-p-ethyl 7.5% EW) as compared to hand weeding were devoted in sub plots. The obtained results revealed that Sakha 108 as Japonica rice cultivar was resistant against tested herbicides and recorded the highest values of rice dry weight, chlorophyll content, number of panicles m^{-2}, panicle weight and grain yield, while scored the lowest values of total phenols. On the other hand, Giza 177 as short duration Japonica rice cultivar showed the higher sensitivity against tested herbicides and achieved the lowest values of abovementioned agronomic traits and chlorophyll content. Cyhalofop-butyl as ACCase herbicide was safer on studied rice cultivars and recorded the highest values of studied agronomic characteristics for rice as well as chlorophyll content, while recorded the lowest values of total phenols during the study. On the other hand, bispyribac-sodium 2% SL resulted in more toxic effect for rice cultivars and recorded the lowest values of studied agronomic traits for rice. fenoxaprop-p-ethyl 7.5% EW achieved the lowest chlorophyll content and highest values of total phenols during the study. Giza 179 as Indica-Japonica rice cultivar appeared high sensitivity when sprayed with bispyribac-sodium and fenoxaprop-ethyl but made recovery after 12 days from herbicidal application and recorded the highest chlorophyll content, dry weight, panicles m^{-2} and grain yield with no significant differences between Sakha 108 rice cultivar during the study. In respect of herbicide residues in rice seeds, all tested herbicides didn't have residues in rice seeds at 100 days after treatment. It means that tested herbicides didn't have any residues in rice seeds.

Keywords: Chlorophyll; cultivars; herbicides; phenols; phytotoxicity; residues; rice and weed control.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a strategic cereal crop for food security worldwide, it is public and main dish for most African and Asian people. Rice is essential crop for Egyptian cultivation; it keeps the balance in the coastal areas beside the Mediterranean Sea (salt belt) from salt erosion by sea water, moreover it considered a reclaimed crop in salt-affected soils in Kafrelsheikh, Alexandria, Beheira, Port Said and Damietta. Rice cultivated area in Egypt was 474,494 hectares which produced 4,841,327 tons of paddy rice with a productivity of 10.2 tons per hectare [1].

Rice farming faces many biotic and abiotic stresses, weeds are the main constrain in rice production system which reduces the quantity and quality of rice seeds and increases production cost consequently reducing net income for farmers. Many weed species are dominant in Egyptian rice fields especially after shifting from transplanted rice to direct seeding system which provided more aggressive weeds to attack rice plants and decreased use efficiency of water, nutrients, sunlight and soil space by rice plants and caused about 17- 47 yield losses in transplanted rice [2] and 93% in direct-seeded rice [3].

Chemical weed control as the first and maybe the last line of defense against weeds is depends on herbicides (chemical substances or biological organisms that kill or reduce weed growth by negative effects on one or more vital processes in the weed and minimize or prevent yield losses of the crop). Herbicides use started in 1200 BC when farmers applied Nacl as a non-selective herbicide against weeds, after this date many herbicide groups were innovated, ACCase inhibitors herbicides such as cyhalofop-butyl and fenoxaprop-ethyl were appeared at 1975, while Aceto Lactate Synthases (ALS) such as bispyribac-sodium and penoxsulam were innovated at 1980, since this date no new active ingredients were introduced.

Herbicides that control grassy weeds must be high selective to kill weeds without any toxicity on rice plants in addition to herbicides must be economical and have no residues in rice seeds. But there are some grasses herbicides that cause phytotoxicity on rice plants such as bispyribac-sodium belonging to Acetolactate synthesis (ALS) inhibitor herbicides, such enzyme is essential for the biosynthesis of Valine, Leucin and isoleucine amino acids [4] and fenoxaprop-ethyl. Toxicity symptoms may be one or more of yellowing leaves, necrosis, brown leaf tips, temporary stunting, reduction in tillering ability and plant kill [5] which established phytotoxicity scale in rice depending on the abovementioned symptoms as follows, 1= no toxicity, 2 = slightly toxicity, 3 = moderately toxicity, 4 = severe toxicity and 5 = plant kill. Kumar et al. [6] reported that fenoxaprop-ethyl caused leaves yellowing, necrosis and stunting at different growth stages of rice. Moreover, herbicide phytotoxicity is higher in direct seeding than transplanted rice because of early post application of certain herbicides [7].

Rice cultivars are different in herbicide sensitivity, generally Japonica rice cultivars more tolerant against herbicides than Indica and Indica/Japonica rice. El-Sobki [8] found that Giza 178 as Indica/Japonica rice cultivar was more sensitive to herbicides than Giza 179, EHR1 and Japonica rice cultivar Sakha 104. There are two defense strategies for plants to face herbicide phytotoxicity (1) enzymatic antioxidant system such as catalase enzyme. (2) Non-enzymatic antioxidant system by releasing one or more of phenolic, proteins or amino acid compounds.

The levels of herbicide residue in rice grains don't have to exceed the maximum residue levels (MRL) and the pre-harvest interval (PHI) according to the European Union (EU) Regulation No. 396/2005 [9,10]. To assure accurate quantification of very low residue levels in food products sensitive methodologies are validated. The modified QuEChERS coupled with (HPLC) connected with photodiode array detector (DAD) analysis technique are much recommended to obtain efficient extraction in addition to clean, easy and cheap quantitation methods of a large number of pesticide residues in rice [11] and are considered one of the most distinctive AOAC official protocols for quantitation [12]. The main target of this study is to differentiate among Egyptian rice cultivars to make a map for herbicidal sensitivity and physiological behavior of these cultivars under the common active ingredients of chemical herbicides in Egypt as well as the residues of these herbicides in rice seeds.

2. MATERIALS AND METHODS

A field study was conducted at the Experimental Farm of Rice Research Department, Sakha Agricultural Research Station, Kafrelsheikh, Egypt during the summer seasons of 2021 and 2022 to study the phytotoxicity of common herbicides against grassy weeds and rice vegetative growth, yield and its attributes as well as herbicides residues in seeds of three Egyptian rice cultivars cultivated by broadcasting method. Seeding dates were 14 and 20 of May during the first and second seasons, respectively. The plot size was 15 m^2 (3 x 5 m) and the rest agricultural practices were done as recommended according to RRTC recommendations [13].

Studied factors: included two factors as follow:

A- Rice cultivars

Three rice cultivars were tested included Giza 177, Giza 179 and Sakha 108. Parentage, duration, type, plant height and year of release of evaluated cultivars are presented in Table 1.

B- Weed control treatments

Four herbicides were applied as recommended for dose, time and method of application compared with hand weeding three times as follows:

1- Penoxsulam (Rainbow 2.5% OD) at the recommended dose (0.0238 kg ai ha^{-1}) at 15 days after seeding (DAS).
2- Bispyribac-sodium (Nominee 2% SL) at the recommended dose (0.0381 kg ai ha^{-1}) at 18-22 DAS.
3- Cyhalofop-butyl (Bazooka 10% EC) at the recommended dose (0.286 kg ai ha^{-1}) at 15-20 days after seeding (DAS).
4- Fenoxaprop-ethyl (Whip-super 7.5% EW) at the recommended dose (0.0625 kg ai ha^{-1}) at 35 DAS.
5- Hand weeding three times (at 20, 40 and 60 DAS).

Four tested herbicides were sprayed using a knapsack sprayer (300 liter water per hectare) on saturated soil, then the field was flooded at 24 hours after herbicide application and the field was kept flooded for three days after treatment.

Table 1. Parentage, duration, type, plant height and year of release for tested rice cultivars

Traits	Giza 177	Giza 179	Sakha 108
Parentage	Giza171/ Yamji No.1// Pi No.4	Gz1368-S-5-4 / Gz6296-12-1-2-1-1	Sakha101 / HR5824-B-3-2-3 // Sakha101
Duration (days)	125	120	135
Type	Japonica	Indica/Japonica	Japonica
Plant height (cm)	100	95	95
Year of release	1995	2012	2018

2.1 Sampling and Data Recorded

A- Physiological traits

Five plants from each plot were randomly sampled to determine total chlorophyll and total phenols before and then after herbicide treatments, one sample was taken every three days. Extraction and determination of photosynthetic pigments was done according to Lichtenthaler and Buschmann [14].

The chemical content of leaves as total chlorophyll content accumulation as mg/g fresh weight of leaves was determined according to Lichtenthaler and Buschmann [14], and total phenol contents (TPC) as mg/g of fresh weight were determined by using the method described by Liyana-Pathirana and Shahidi [15].

B- Vegetative growth for weeds

At 30 days after herbicidal application, an area of 0.5 x 0.5 m was sampled and repeated four times from each plot then air dried, after dried in the oven at 70 °C for 48 hours or to stable weight then dry weight of the total weeds was recorded and converted to g m^{-2}.

C- Vegetative growth, yield and its attribute of rice crop

For rice dry weight, it was determined using the same technique of weeds at 30 days after herbicidal application. Before harvest, plant height (cm) was measured for ten plants from each plot and panicles were counted in four replicates by area of 0.5 m x 0.5 m then converted number of panicles per square meter. Ten panicles of rice plants were randomly sampled before harvest from each plot to determine panicle weight, 1000-grain weight and filled grains per panicle. After rice maturity, four square meters from the plot center were harvested to determine grain yield while adjusted at 14% moisture content then converted to tons ha^{-1}.

D- Sampling, extraction, and clean-up

Homogenization of rice grain (100 grams for each sample) was carried out using a food processor (Thermomix, Vorwerk) for five minutes at high speed in a laboratory. The homogenate of each sample was then placed into 50-ml polypropylene centrifuge tubes and frozen at −20 °C until further analysis. Extraction takes place according to the procedure described and modified by Lehotay *et al*. [16]. Five grams of each homogenized sample was weighed into a 50-ml centrifuge tube and 10 ml water. Extraction and cleaning up were done by an optimized QuEChERS method [17] by blending with ten milliliters of 1.0% acidified acetonitrile with acetic acid and shaking vigorously for one minute using a vortex mixer at maximum speed. Afterward, four grams of anhydrous MgSO4, one gram of NaCl, one gram of sodium citrate dihydrate, and 0.5 g disodium hydrogen citrate sesquihydrate were added, then extracted by shaking vigorously on vortex for two minutes and centrifuged for ten minutes at 3000 rpm. An aliquot of one milliliter was transferred from the supernatant to a new clean five-milliliter centrifuge tube and cleaned by dispersive solid-phase extraction with 25 mg of PSA and 150 mg of magnesium sulfate. Afterward, centrifugation was carried out at 5,000 rpm for 5 min. An aliquot (2 ml) from the supernatant was filtered through a 0.2-µm PTFE filter (Millipore, USA) and then analyzed by Agilent 1100 HPLC-DAD.

2.2 Residue Analysis of Herbicides

The analysis of active ingredients of the tested herbicides (penoxsulam, bispyribac-sodium, cyhalofop-butyl and fenoxaprop-P-ethyl) was performed using a high-performance liquid chromatography system (HPLC) (USA), with a quaternary pump, manual injector (Rheodyne), thermostat compartment for the column, and photodiode array detector. The separation was achieved on a chromatographic column ODS H optimal (150 mm × 4.6 mm, 5 µm film thicknesses). The column was kept at room temperature. Residues were estimated by the comparison of peak area of standards with that of the unknown or spiked samples run under identical conditions the mobile phase, flow rate, and the detection wavelength of penoxsulam, bispyribac-sodium, cyhalofop-butyl and fenoxaprop-P-ethyl were summarized in Table 2.

2.3 Statistical Analysis

Data were subjected to appropriate statistical analysis of variance as reported by Snedecor and Cochran [18]. Data of weeds were transformed according to square-root transformation ($\sqrt{[x} + 0.5]$) then MSTATC program was used to analyze data. Rice data were directly analyzed by same program. Duncan´s Multiple Range Test [19] was used to compare means of weeds and rice traits.

Table 2. Mobile phase, flow rate and detection wave length of studied herbicides

Herbicide	Mobile phase	Flow rate	Detection wave length (nm)
Penoxsulam	(acetonitrile/water) (70:30,v/v)	1 ml/min	230
Bispyribac-sodium	acetonitrile :water (50/50) (v/v)	0.8ml/min	240
Cyhalofop-butyl	methanol: water (75/25) (v/v)	1 ml/min	250
Fenoxaprop-P-ethyl	methanol: water (75/25) (v/v)	1 ml/min	238

HPLC system conditions for detecting Penoxsulam, bispyribac-sodium, cyhalofop-butyl and fenoxaprop-P-ethyl.

3. RESULTS AND DISCUSSION

3.1 Total Photosynthetic Pigments Content on Leaves

Effect of cultivars and weed control treatments and their interaction on total photosynthetic pigments at 3, 6, 9, 12 and 15 days after herbicide application on rice plants during 2021 and 2022 seasons are shown in Table 3. Giza 179 rice cultivar recorded the highest mean value of total photosynthetic pigments at all periods after herbicide treatments compared to Sakha 108 and Giza 177 rice cultivars. Obtained data explain that herbicides caused significant decrease in total photosynthetic pigments at 3, 6, 9, 12 and 15 days after treatments as compared to hand weeding / no herbicides during both seasons of study as shown in Table 3. Hand weeding (without chemicals) produced the highest chlorophyll content than chemical control at all sampling dates during 2021 and 2022 seasons. Cyhalofop-butyl and penoxsulam ranked second in total chlorophyll content, while the lowest values were recorded by fenoxaprop-p-ethyl in both seasons of study.

These results are in harmony with Ahmad et al. [20], Saleem et al. [21] and Hanci and Cebeci [22] who suggested that in total photosynthetic pigments contents usually decrease and reduces in the plants exposed to environmental stresses. Also, Kumar [23] who showed that, the application of herbicides (2, 4-D and IPU) was able to reduce total photosynthetic pigments in plant. Butachlor slightly affected rice leaves in total photosynthetic pigments and amino acids and although exhibited phytotoxic effects on plants, but, the symptoms disappeared 14 DAS [24]. The rate of total photosynthetic pigments is affected adversely due to the inactivation of total photosynthetic pigments [25].

According to the interaction between cultivars and weed control, it is interesting to mention that, Giza 179 cv produced the highest total photosynthetic pigments content when weeds were removed by hand three times during growth season as compared to other cultivars. In addition, Giza 179 chlorophyll contents were decreased after foliar application of herbicides directly then started to increase until reached complete recovery at 12 days after herbicides application. Contrary to this, Giza 177 cv was the most negatively affected cultivar by herbicides where, the lowest values for total photosynthetic pigments were achieved by foliar application of fenoxaprop-p-ethyl herbicide. Sakha 108 as Japonica rice cultivar appeared stable and less injury by herbicides application at all sampling dates during the study. On other hand, Giza179 cv. significantly had higher plant pigments and photosynthesis comparing to other varieties; Giza177 and Sakha 108 [26]. Giza 179 cv. achieved the highest photosynthetic pigments content (Chl. a & b and Carotenoids) as compared to Giza 177 cv. [27] physiological components, photosynthetic pigments; chlorophyll a, chlorophyll b and total chlorophyll (a + b), carotenoids, as well as, total carbohydrates in rice plants are shown in Table 3 and Fig. 1.

3.2 Essential Total Phenols

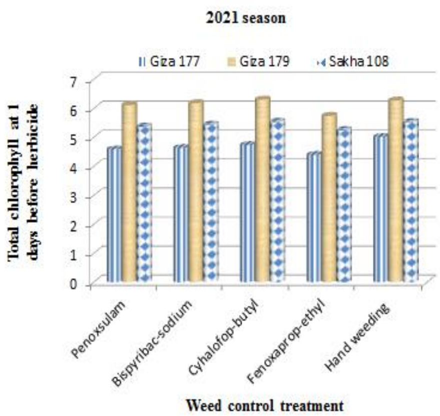
Effects of cultivars and herbicides and their interaction on total phenols at 3, 6, 9, 12 and 15 days after spraying with herbicide treatments in 2021 and 2022 seasons in rice plants are shown in Table 4.

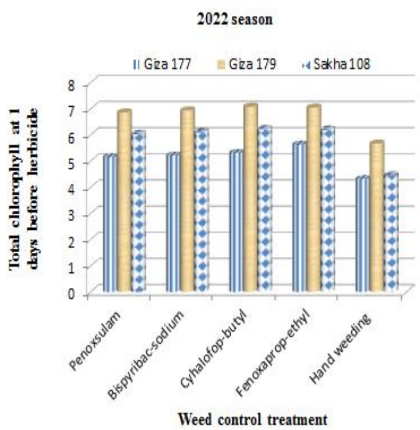
The obtained results showed that rice cultivars significantly differed in total phenols content before and after herbicide treatments. Giza 179 ranked first either before or after herbicidal treatment until 9 DAT. On the other side Giza 177 was the least before treatment, while showed higher phenols content after herbicide treatment by 6 days until 15 DAT. Sakha 108 rice cultivar recorded the lowest values in this respect. The differences in responses for rice cultivars in phenol content due to herbicidal treatments may relate to their genetic background.

Table 3. Total chlorophyll content of rice cultivars before and after herbicides treatment during 2021 and 2022 seasons

Factor	Total chlorophyll					
	1 DBT	3 DAT	6 DAT	9 DAT	12 DAT	15 DAT
	2021 season					
A- Rice cultivars						
Giza 177	4.667 c	4.390 c	4.477 c	4.924 c	5.582 c	6.159 b
Giza 179	6.117 b	5.054 a	5.378 a	5.794 a	6.295 a	6.714 a
Sakha 108	5.426 a	4.898 b	5.054 b	5.380 b	5.725 b	6.296 b
F. test	**	**	**	**	**	**
B-Weed control						
Penoxsulam	5.352 b	4.765 b	4.977 b	5.434 b	6.041 b	6.616 b
Bispyribac-sodium	5.415 b	4.333 c	4.523 c	4.939 c	5.491 c	6.019 c
Cyhalofop-butyl	5.523 a	4.824 b	5.040 b	5.502 b	6.113 b	6.694 ab
Fenoxaprop-ethyl	5.126 c	4.156 d	4.336 d	4.740 d	5.280 d	5.787 d
Hand weeding	5.600 a	5.824 a	5.972 a	6.213 a	6.412 a	6.834 a
F. test	**	**	**	**	**	**
Interaction (A x B)	*	**	**	**	*	**
	2022 season					
A- Rice cultivars						
Giza 177	5.121 c	4.072 b	4.725 c	5.870 c	6.071 a	6.067 b
Giza 179	6.721 a	4.680 a	5.805 a	6.393 a	6.529 a	6.566 a
Sakha 108	5.810 b	4.582 a	5.435 b	6.131 b	6.304 a	6.319 ab
F. test	**	**	**	**	Ns	*
B-Weed control						
Penoxsulam	6.017 b	4.561 b	5.186 c	6.116 c	6.212 c	6.280 c
Bispyribac-sodium	6.087 bc	4.149 c	5.142 d	5.931 d	6.153 c	6.125 d
Cyhalofop-butyl	6.209 ab	4.619 b	5.493 b	6.426 b	6.527 b	6.636 b
Fenoxaprop-ethyl	6.296 a	3.980 d	4.893 e	5.574 e	5.824 d	5.792 e
Hand weeding	4.811 d	4.915 a	5.896 a	6.609 a	6.790 a	6.754 a
F. test	**	**	**	**	**	**
Interaction (A x B)	*	**	**	**	**	**

*** indicates P< 0.01, * indicates P< 0.05. In a season, means of each factor within each column, values followed by the same letter are not significantly differed at 5% level, using DMRT. DBT= days before treatment, DAT= days after treatment.*





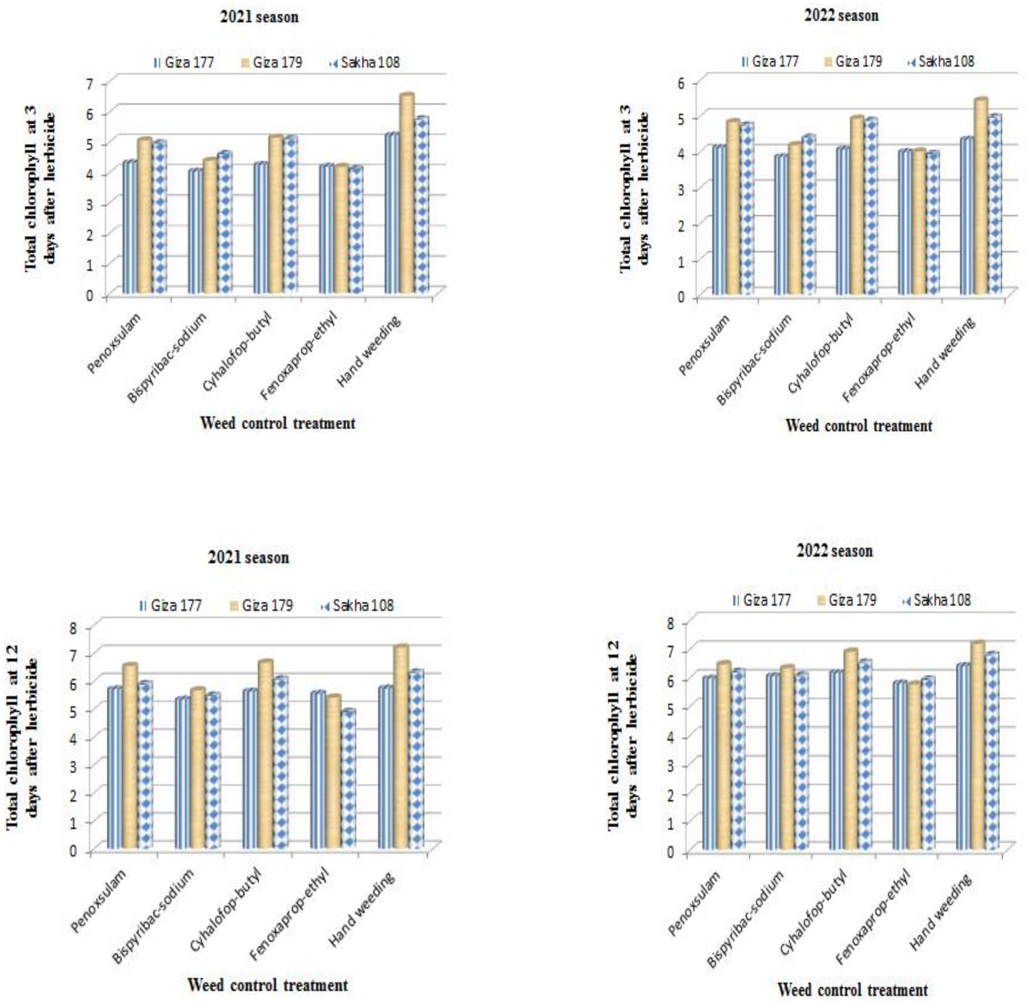


Fig. 1. Effect of interaction between herbicides treatments and cultivars on total photosynthetic pigments in 2021 and 2022 seasons

Application of penoxsulam, bispyribac-sodium, cyhalofop-butyl and fenoxaprop-p-ethyl herbicides caused a significant increase in total phenols in rice plants are shown in Table 4. The lowest values for total phenols were recorded by hand weeding / no chemicals followed by foliar application of penoxsulam and cyhalofop-butyl compared with bispyribac-sodium and fenoxaprop-ethyl during two seasons of study. These results may be due to the effect of fenoxaprop-p-ethyl inhibition of acetoacetate synthase (ALS) which is responsible about biosynthesis of the branched chain catalase such as total phenols [28].

Concerning the influence of interaction between weed control treatments and rice cultivars on total essential phenols, the results revealed at 3 DAT both of Giza 177 and Giza 179 treated with fenoxaprop-p-ethyl as well as Giza 179 treated with bispyribac-sodium recorded the highest total phenols during the study. While at 12 DAT, Giza 179 as Indica-Japonica rice cultivar appeared complete recovery under treatments of bispyribac-sodium and fenoxaprop-p-ethyl. The lowest content of total phenols was obtained by Sakha 108 Japonica rice cultivar when treated with cyhalofop-butyl with no significant differences between the same cultivar under hand weeding during the study. It is important to note that, the Giza 177 cv. was the most varieties severely affected by herbicides, while Giza 179 rice cultivar was sensitive against both of bispyribac-sodium and fenoxaprop-p-ethyl but started recovery at 9 DAT and completely recovered at 12 DAT. Sakha 108 cultivar appeared more tolerant against all tested herbicides during the study. It means that tested rice cultivars can be sprayed with the four studied herbicides with no harmful effect on chlorophyll content and total phenols. While Giza 177 can safely treated with penoxsulam and cyhalofop-butyl and avoiding application of both bispyribac-sodium and fenoxaprop-p-ethyl with such cultivar.

However, the lowest values for total essential phenols were achieved by Sakha 108 rice cultivars x hand weeding / no herbicides during 2021 and 2022 seasons. Similar significant effects were reported by Zarzecka and Gugala [29] who found that herbicides can have certain effects on changes in plant physiological processes. Souahi et al. [30] stated that, herbicides affected all aspects of primary and secondary.

3.3 Effect of Studied Factors on Dry Weights of Total Weeds

Data presented in Table 5 showed that Giza 179 as Indica-Japonica rice cultivar reduced dry weight of accompanied weeds and recorded the lowest values of total weeds during 2021 and 2022 seasons. While Giza 177 as Japonica rice cultivar achieved the highest dry weight of total weeds during the study. These results may be due to high tillering ability and rapid growth rate of Giza 179 resulted in high competitive ability against weeds [31].

Table 4. Total phenols of rice cultivars before and after herbicides treatment during 2021 and 2022 seasons

Factor	Total phenols					
	1 DBT	**3 DAT**	**6 DAT**	**9 DAT**	**12 DAT**	**15 DAT**
	2021 season					
A- Rice cultivars						
Giza 177	1.008 c	2.085 b	2.115 a	2.067 a	1.956 a	1.809 a
Giza 179	1.331 a	2.326 a	2.263 a	2.087 a	1.771 b	1.732 b
Sakha 108	1.172 b	2.400 a	1.895 b	1.791 b	1.739 b	1.657 c
F. test	**	*	**	*	*	*
B-Weed control						
Penoxsulam	1.166 b	2.393 c	2.098 b	1.979 c	1.771 c	1.648 c
Bispyribac-sodium	1.171 b	2.548 b	2.246 a	2.113 b	1.877 b	1.717 bc
Cyhalofop-butyl	1.170 b	2.376 c	2.098 b	1.976 c	1.759 c	1.660 c
Fenoxaprop-ethyl	1.213 a	2.670 a	2.349 a	2.212 a	1.968 a	1.859 a
Hand weeding	1.132 c	1.365 d	1.662 c	1.629 d	1.736 c	1.779 b
F. test	**	**	**	**	**	**
Interaction (A x B)	**	**	**	**	**	**
2022 season						
A- Rice cultivars						
Giza 177	1.129 c	2.335 b	2.369 a	2.315 a	2.191 a	2.026 a
Giza 179	1.491 a	2.605 a	2.534 a	2.338 a	1.984 b	1.940 ab
Sakha 108	1.313 b	2.688 a	2.122 b	2.006 b	1.948 b	1.855 b
F. test	**	**	**	**	**	**
B-Weed control						
Penoxsulam	1.311 b	2.681 c	2.350 b	2.216 b	1.983 c	1.846 c
Bispyribac-sodium	1.306 b	2.854 b	2.516 a	2.367 a	2.103 b	1.923 b
Cyhalofop-butyl	1.311 b	2.661 c	2.350 b	2.213 b	1.970 c	1.859 c
Fenoxaprop-ethyl	1.358 a	2.991 a	2.631 a	2.477 a	2.205 a	2.082 a
Hand weeding	1.268 c	1.528 d	1.862 c	1.825 c	1.945 c	1.992 c
F. test	**	**	**	**	**	**
Interaction (A x B)	**	**	**	**	**	**

*** indicates P< 0.01, * indicates P< 0.05. In a season, means of each factor within each column, values followed by the same letter are not significantly differed at 5% level, using DMRT. DBT= days before treatment, DAT= days after treatment.*

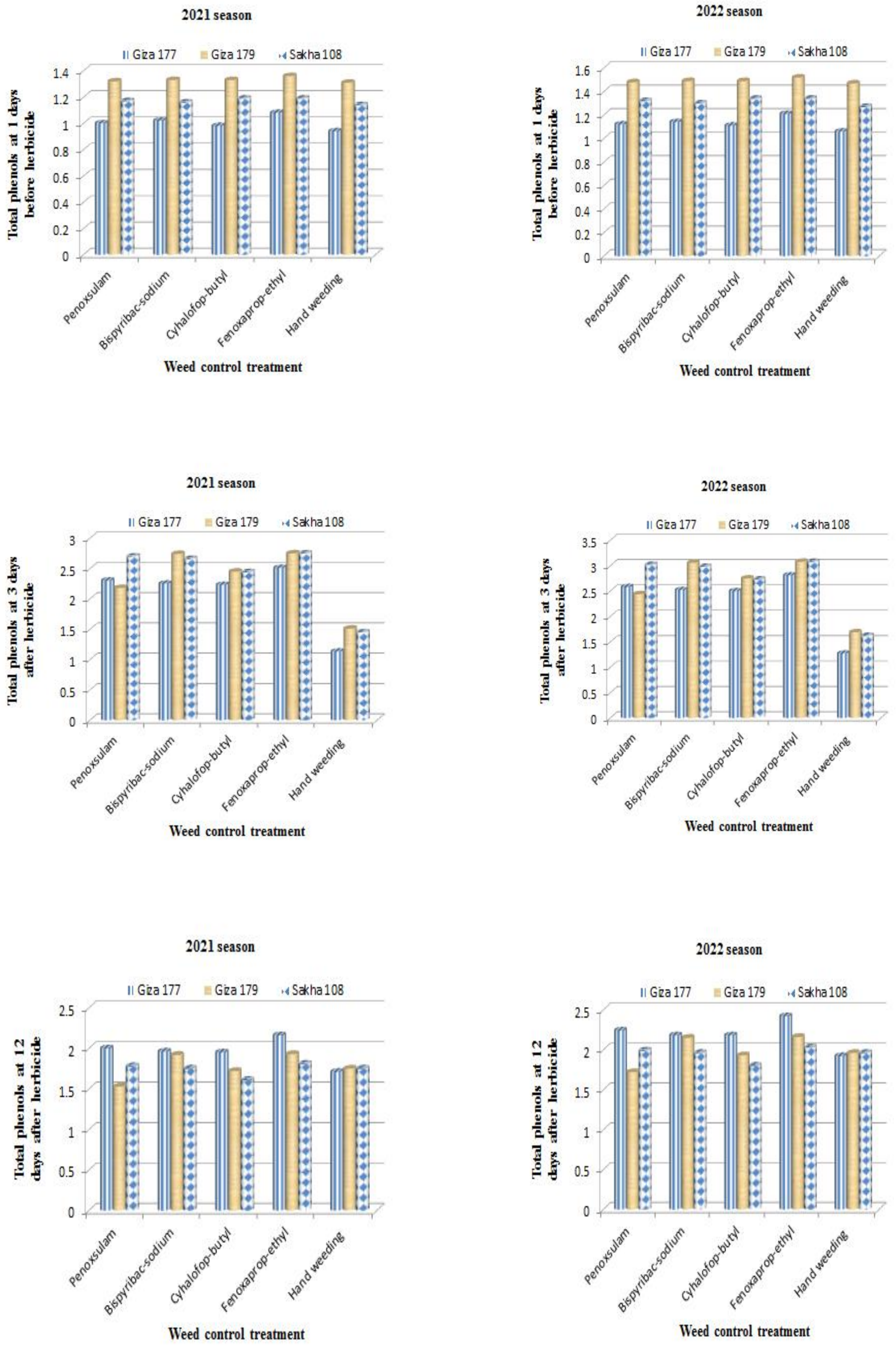


Fig. 2. Effect of the interaction between herbicides treatments and cultivars on total essential phenols in 2021 and 2022 seasons

Table 5. Effect of rice cultivars and weed control treatments on dry weights of total weeds during 2021 and 2022 seasons

Factors	Dry weight of total weeds (g m^{-2})			
	Original data		Transformed data	
	2021	2022	2021	2022
A-Rice cultivars				
Giza 177	176.3	116.3	13.2 a	10.7 a
Giza 179	119.3	90.7	10.8 c	9.4 b
Sakha 108	137.3	99.8	11.6 b	9.9 b
F. test			**	**
B-Weed control				
Penoxsulam	151.8	121.3	12.3 b	11.0 b
Bispyribac-sodium	136.0	99.2	11.6 c	10.0 c
Cyhalofop-butyl	116.0	74.2	10.7 d	8.6 d
Fenoxaprop-ethyl	100.2	66.9	10.0 e	8.2 e
Hand weeding	217.6	149.9	14.7 a	12.2 a
F.test	-	-	**	**
Interaction (A x B)	-	-	NS	NS

*** indicates P< 0.01, NS= not significant. For transformed values, in a column, means of transformed data followed by the same letter are not significantly different at 5% level, using Duncan's Multiple Range Test.*

For weed control treatments, data in Table 5 showed that all chemical weed control treatments exceeded hand weeding and decreased the dry weight of total weeds in broadcast-seeded rice during the study. Fenoxaprop-ethyl recorded the lowest values of total weeds during the 2021 and 2022 seasons, while cyhalofop-butyl 10% EC ranked second in this respect. Hand weeding achieved the higher dry weight of accompanied weeds during the study. These results reflect the high efficiency of ACCase herbicides such as fenoxaprop-ethyl and cyhalofop-butyl in inhibition of acetyl-CoA carboxylase then negatively affect fatty acid synthesis and consequently inhibits phospholipids production which is necessary for cell growth [32].

3.4 Effect of Studied Factors on Agronomic Characteristics of Rice

3.4.1 Effect of rice cultivars on plant height, dry weight and number of panicles per square meter of rice

Data presented in Table 6 showed that there were significant differences among tested rice genotypes during 2021 and 2022 seasons. Giza 177 rice cultivar which was released in 1997 as short-duration japonica rice [33] recorded the highest values for plant height as average during both seasons followed by Sakha 108 japonica rice cultivar, while Giza 179 as Indica-Japonica short duration rice cultivar recorded the lowest values in this respect. It may be reflecting the genetic background of Giza 177 which is the tallest plant type among tested rice cultivars under normal conditions.

For rice dry weight and number of panicles, Sakha 108 rice cultivar was released in 2018 [34] produced the highest dry biomass and number of panicles m^{-2} during both seasons of study. Giza 179 as Indica-Japonica rice cultivar ranked second in both characteristics during 2021 and 2022 seasons. These results explain the high tillering ability of Sakha 108 as medium-duration rice cultivar (135 days) consequently producing high dry biomass during the growth season as compared to short-duration rice cultivars Giza 177 and Giza 179.

3.4.2 Effect of weed control treatments on plant height, dry weight and number of panicles m^{-2} of rice

It could be observed from the data presented in Table 6 that all weed control treatments significantly affected the abovementioned characteristics during the study. Cyhalofop-butyl appeared more safety on growth of tested rice cultivars and produced the highest values of plant height, dry weight and number of panicles m^{-2} of rice with no significant differences with penoxsulam during the second season of study for plant height and dry weight of rice. Bispyribac-sodium active ingredient of Nominee 2% caused the highest decrease in plant height (cm), dry weight (g) and effective tillers m^{-2} during 2021 and 2022 seasons.

Table 6. Plant height, dry weight and number of panicles of rice as influenced by rice cultivars and weed control treatments during 2021 and 2022 seasons

Factors	Plant height (cm)		Rice dry weight (g m^{-2})		Rice number of panicles (m^{-2})	
	2021	2022	2021	2022	2021	2022
A-Rice cultivars						
Giza 177	91.0 a	94.4 a	998.6 c	1087.8 c	452.3 c	453.3 c
Giza 179	86.9 c	88.5 c	1156.9 b	1220.1 b	528.0 b	552.5 b
Sakha 108	89.7 b	90.2 b	1206.6 a	1306.3 a	554.7 a	610.1 a
F. test	**	**	**	**	**	**
B-Weed control						
Penoxsulam	91.0 b	93.1 a	1164.5 b	1304.9 a	504.9 b	552.9 b
Bispyribac-sodium	85.5 c	88.3 c	1041.6 d	1104.8 c	506.7 b	512.c
Cyhalofop-butyl	92.4 a	93.1 a	1233.2 a	1311.7 a	554.7 a	572.4 a
Fenoxaprop-ethyl	85.1 c	89.8 b	1123.5 c	1196.7 b	520.9 b	565.3 ab
Hand weeding	91.8 ab	90.8 b	1040.7 d	1105.6 c	471.0 c	490.7 d
F. test	**	**	**	**	**	**
Interaction (A x B)	**	**	**	**	**	**

*** indicates P< 0.01. Means of each factor within each column, values followed by the same letter are not significantly differed at 5% level, using DMRT.*

Table 7. Effect of rice cultivars and weed control treatments on panicle weight and grain yield of rice during 2021 and 2022 seasons

Factors	Rice panicle weight (g)		Rice grain yield (t ha^{-1})	
	2021	2022	2021	2022
A-Rice cultivars				
Giza 177	1.9 c	1.9 c	7.741 b	8.045 b
Giza 179	2.1 b	2.2 b	8.995 a	9.201 a
Sakha 108	2.4 a	2.4 a	8.855 a	9.403 a
F. test	**	**	**	**
B-Weed control				
Penoxsulam	2.2 b	2.2 b	8.279 c	8.823 c
Bispyribac-sodium	2.1 c	2.1 c	8.461 c	8.965 bc
Cyhalofop-butyl	2.3 a	2.4 a	9.537 a	9.775 a
Fenoxaprop-ethyl	2.1 b	2.3 b	8.960 b	9.214 b
Hand weeding	1.9 c	2.1 c	7.414 d	7.637 d
F. test	**	**	**	**
Interaction (A x B)	**	**	*	**

*** indicates P< 0.01, * indicates P< 0.05. Means of each factor within each column, values followed by the same letter are not significantly differed at 5% level, using DMRT.*

Bond *et al*. [35] stated that bispyribac-sodium as ALS (acetolactate synthesis inhibitor) herbicide caused plant stunting, decreased tillering ability of rice which resulted in low dry biomass. Scasta et al. [36] found that bispyribac-sodium caused 30% injury for rice plants and can increase by increasing the dose of this herbicide. The superiority of cyhalofop-butyl as a synthetic ACCAse inhibitor may be due to the high efficiency of such an active ingredient in controlling weeds with no harmful effect on tested cultivars of Egyptian rice. Anwar *et al*. [37] reported that cyhalofop-butyl had phytotoxicity rate by one at 7, 14 and 21 days after application. It means that this herbicide causes very slight injury/no toxicity on rice plants.

3.4.3 Effect of rice cultivars on panicle weight and grain yield of rice

As shown from the data in Table 7, it is evident that rice cultivars significantly differed in panicle weight and grain yield of rice during 2021 and 2022 seasons. Japonica rice cultivar Sakha 108 produced the heaviest panicle weight in addition to the highest grain yield during both seasons of study, while Giza 179 ranked second in this respect during the study. The superiority of Sakha 108 rice cultivar may be due to high yielding capacity by producing more panicles as well as heavy panicle weight which gives high grain yield [34].

3.4.4 Effect of weed control treatments on panicle weight and grain yield of rice

Data included in Table 7 showed the significant effect of tested weed control treatments on panicle weight and grain yield of rice during the study. Cyhalofop-butyl as ACCase inhibitor herbicide produced the highest values of panicle weight and grain yield of rice during 2021 and 2022 seasons. Fenoxaprop-ethyl active ingredient ranked second in panicle weight and grain yield of rice during the study. Bispyribac-sodium ranked third because of injury caused against rice plants. On the other hand, the lowest values of panicle weight and grain yield were obtained by hand weeding under broadcast-seeded rice during the 2021 and 2022 seasons. These results may be due to the high efficiency of ACCase inhibitors herbicides in killing weeds, moreover saving nutrients, water space and sunlight for rice plants without competition caused by weeds, in addition to slight injury / no toxicity for cyhalofop-butyl on Giza 177, Giza 179 and Sakha 108 rice cultivars during growth season.

3.5 Interaction Effect on Agronomic Traits of Rice

Data on plant height (cm), dry weight (g m^{-2}), number of panicles m^{-2}, panicle weight (g) and grain yield of rice (t ha^{-1}) as influenced by the interaction between cultivars and weed control treatments in 2021 and 2022 seasons:

Rice dry weight (g m^{-2}) was considerably influenced by the interaction of rice cultivars and weed control treatments during 2021 and 2022 seasons. (Fig. 3). It is clear from the figure that Sakha 108 cultivar showed its superiority in dry weight followed by Giza 179 under all weed control treatments, while Giza 177 rice cultivar showed the least. The lowest dry weight of rice was obtained from Giza 177 cultivar sprayed with bispyribac-sodium while the heaviest one was exhibited by Sakha 108 when treated by cyhalofop-butyl. The reduction of dry weight of Giza 177 rice cultivar under fenoxaprop-ethyl application may refer to the sensitivity of this cultivar to such herbicide while both Giza 179 and Sakha 108 cultivars were more tolerant in this respect. Griffin and Baker [38] recorded different injury degrees due to the application of fenoxaprop and haloxyfop herbicides.

As shown in Fig. 4, rice plant height was affected by the interaction of cultivars and weed control treatments. Giza 179 showed sensitivity against bispyribac-sodium and fenoxaprop-ethyl herbicides especially at 3, 6, 9 and 12 days after herbicide application and recorded the shortest plant height followed by Sakha 108 rice cultivar, while Giza 177 rice cultivar exhibited the tallest plants in this respect. On the other hand, cyhalofop-butyl treatment recorded the tallest rice plants for Giza 177 cultivar. Lanclos *et al*. [39] found that Glufosinate application reduced plant height of CPRS P-B -13 cultivar. Griffin and Baker [38] found that rice cultivars showed stunting and stand ass dye to the application of fenoxaprop-ethyl at post-flood.

As shown in Fig. 5, number of rice panicles/m^2 was greatly affected by cultivars x weed control treatments interaction in both seasons of the study. The highest number of panicles/m^2 was recorded by Sakha 108 cultivar followed by Giza 179 cultivar under all weed control treatments. The lowest number of panicles per unit area was observed by Giza177 cultivar when treated by fenoxaprop-ethyl. The same behavior was observed during 2021 and 2022 seasons. The reduction in panicle number of Giza 177 under fenoxaprop-ethyl treatment could be due to the depression in dry weight accumulation by this cultivar under this treatment as a result of its sensitivity to fenoxaprop-ethyl treatment. These results are in agreement with those obtained by Griffin and Baker [38].

The panicle weight of rice as influenced by the interaction between rice cultivars and weed control treatments is shown in Fig. 6. It is clear from this figure that this interaction clearly affected panicle dry weight of rice cultivars during the two seasons of the study. The best weed control treatment was cyhalofop-butyl which produced the heaviest panicles of rice than other treatments. Under all treatments of weed control, Sakha 108 showed its superiority followed by Giza 179 in this respect. The lowest weight in rice panicle was observed in Giza 177 cultivar under fenoxaprop-ethyl application. This result may be due to the varietal sensitivity to such herbicide as mentioned by Zhang et al. [40].

As shown in Fig. 7, rice grain yield was influenced by the interaction between rice cultivar and weed control treatments during 2021 and 2022 seasons. Sakha 108 cultivar achieved the highest grain yield followed by Giza 179 cultivar under all weed control treatments, while Giza 177 was the least. The lowest grain yield was noticed in Giza 177 when treated by fenoxaprop-ethyl or hand weeding. The inferiority of Giza 177 cultivar in grain yield under fenoxaprop application may explain the high varietal sensitivity to this chemical ingredient than other tested rice cultivars. Willingham et al. [41], Bond and walker [4] reported different varietal tolerances in rice cultivars due to different applied chemical treatments [42].

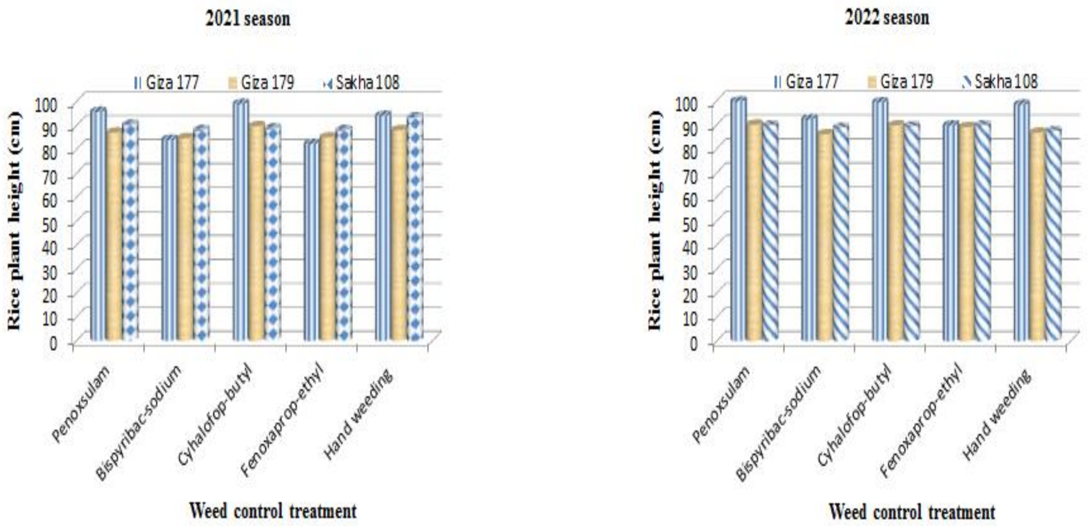


Fig. 3. Rice plant height (cm) as affected by the interaction between rice cultivars and weed control treatments in 2021 and 2022 seasons

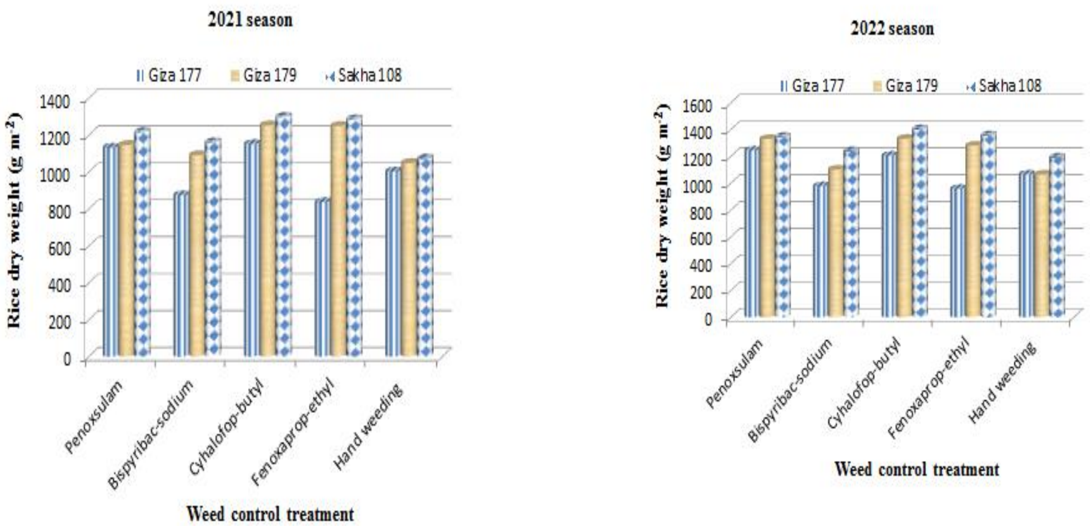


Fig. 4. Rice dry weight (gm^{-2}) as affected by the interaction between rice cultivars and weed control treatments during 2021 and 2022 seasons

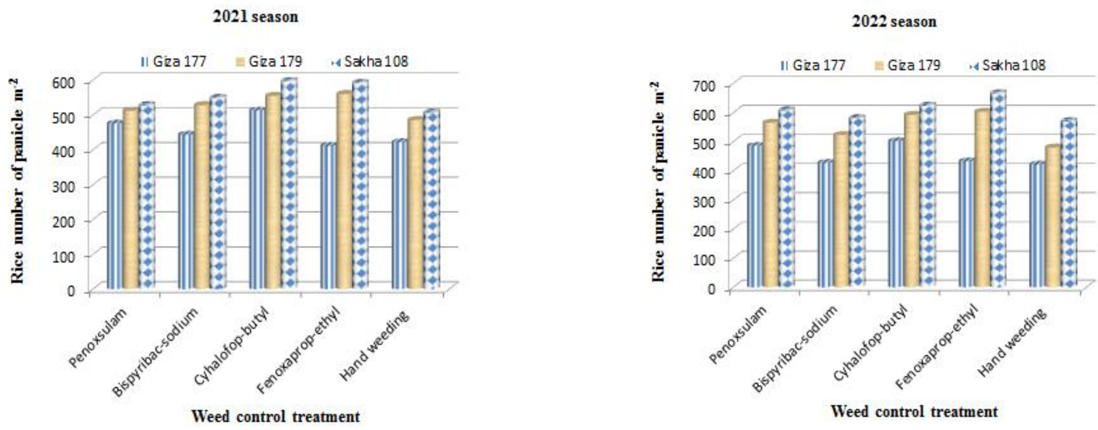


Fig. 5. Rice number of panicles (cm) as affected by the interaction between rice cultivars and weed control treatments during 2021 and 2022 seasons

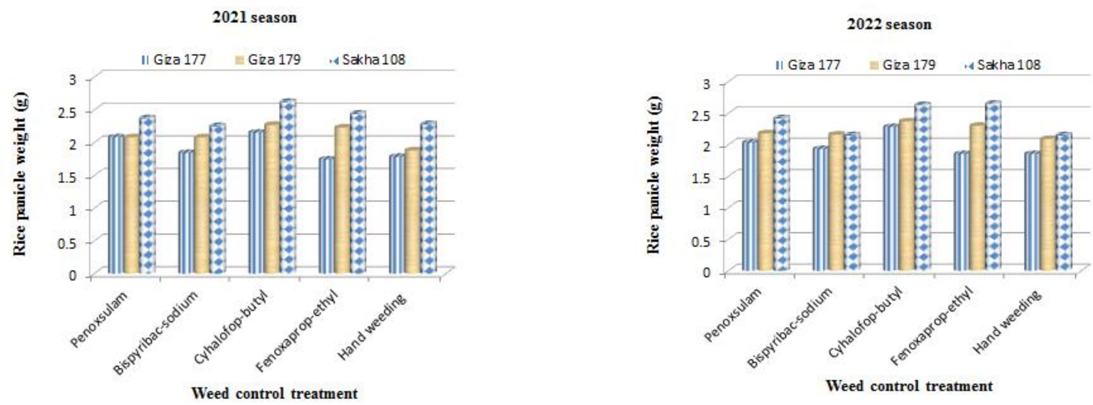


Fig. 6. Rice panicle weight (g) as affected by the interaction between rice cultivars and weed control treatments during 2021 and 2022 seasons

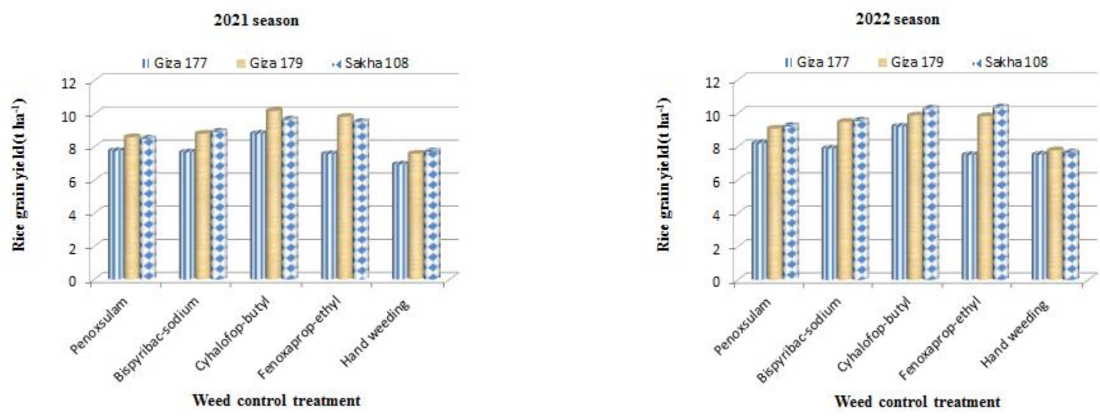


Fig. 7. Rice grain yield (t ha^{-1}) as affected by the interaction between rice cultivars and weed control treatments during 2021 and 2022 seasons

Table 8. Determination of penoxsulam, bispyribac-sodium, cyhalofop-butyl and fenoxaprop-p-ethyl in/on rice seeds at 100 days after application

Interval days	Residues (mg/kg)			
	Penoxsulam	Bispyribac-sodium	Cyhalofop-butyl	Fenoxaprop-p-ethyl
100	ND	ND	ND	ND
MRL(EU)	0.02 mg/kg	0.02 mg/kg	0.01 mg/kg	0.01 mg/kg
RECOVERY	94.22 %	114.26 %	90 %	108.97 %
LOQ	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg
PHI (days)	100			

MRL= acceptable maximum residue limit, LOQ= limit of quantification. PHI= pre-harvest interval. ND= not detected.

3.6 Residue of Herbicides in Rice Seeds

As shown from data presented in Table 8, pre-harvest interval PHI of the penoxsulam, bispyribac-sodium, cyhalofop-butyl and fenoxaprop-p-ethyl reverse-phase (HPLC) analysis were used. PHI values were 100 days for all of them and these results were in agreement with Tsochatzis et al. [43] and Jaiswal et al. [44]. MRL values ranged between (0.01 and 0.02 mg/kg) that were below the MRLs established for fenoxaprop-p-ethyl, cyhalofop-butyl and bispyribac-sodium respectively and these results were in agreement with Menon [45] and Sekhar et al. [46]. Good recoveries between 90 and 114% were obtained during method development. The recovery results matched with the EU method validation guidelines reported for rice [47].

4. CONCLUSION

Sakha 108 Japonica rice cultivar appeared resistant against four tested herbicides and recorded the highest chlorophyll content as well as produced the highest grain yield, while Giza 179 as Indica-Japonica rice cultivar showed sensitive against the application of bispyribac-sodium and fenoxaprop-ethyl but made complete recovery after 12 days from herbicidal application and recorded the highest grain yield with no significant differences between Sakha 108. On the other hand, Giza 177 was more sensitive against both fenoxaprop-ethyl and bispyribac-sodium and produced the lowest chlorophyll content as well as grain yield and achieved the highest total phenols content when treated by both herbicides. Penoxsulam and cyhalofop-butyl were safe on three rice cultivars but cyhalofop was more effective against weeds and produced the highest chlorophyll content and grain yield. Fenoxaprop-ethyl and bispyribac-sodium caused phytotoxicity on Giza 177 and Giza 179 rice cultivars during the study. Analysis of active ingredients for tested herbicides proved that no any herbicide residues were found in rice seeds after 100 days from herbicides application in seeds of studied rice cultivars, which means application of these herbicides in rice cultivation is safe for human consumption and health.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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