Evaluating oxidative activity and flavonoids content of four varieties of oat

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Abstract:

A field experiment was conducted during winter of 2022-2023 in one of the fields at the Shumali region in Babylon Governorate, within latitude of 32.32˚ north and 44.92˚ east. The study planned to evaluate oxidative activities and flavonoid content to four varieties of oats (Shifaa, Oat11, Algouda, and Carlup). The experiment was carried out according to randomized complete block design with three replications, All data were collected and analyzed according to analysis of variance table. The means were tested according to the least significant difference test at 0.05 probability. The results showed significant superiority of the Algouda variety in plant height (127 cm), flag leaf area (27 $cm^{2)}$, and capacity of total antioxidant (83.8 mg g^{-1} dry weight). Total flavonoids (163.8 mg kg^{-1} dry weight), free radicals inhibition efficacy in grains (72.58%), and the grain content of some Flavonoid compounds. Shifaa variety was significantly superior in the number of tillers (478.1 tillers m^{-2}), number of spikes (457.4 spikes m^{-2}), weight of thousand grains(36.97 g), and the grains yield (7.15 tons ha^{-1}).

Keywords: Oats, Varieties, Flavonoids, Yield.

1- Introduction

Oat plant Scientific name (*Avena sativa* L.) is classified under the Poaceae family. It is ranked seventh among crops in terms of importance and productivity. The global production of oat reached 22571618 tons, with a cultivated area estimated 9562497 hectares. Russia ranks first in the world in its cultivation [1], interest has increased at the present time due to its many beneficial effects in the community health. This including cancer prevention, weight reduction, contributes to determining harmful levels and high blood pressure [2]. Each 100 grams of oats contains 14.5% protein, 10.5% fat, 2.27% Fiber, 60.7% carbohydrates, potassium 379 mg / 100 grams [3]. It is also used as a multi-purpose product in industrial and pharmaceutical foods and animal feed. Oat cultivation in Iraq is still limited due to farmers lack of knowledge about the requirements for its cultivation and importance. Therefore, intensive studies must be conducted on this crop in Iraq related to study the effect of varieties and some agricultural operations on growth

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characteristics, yield and its components, and knowing the differences between oat varieties and the extent of their interaction with the environment [4].

Oat grains contain antioxidant compounds, the most important of which are phenolic and flavonoids compounds [5], which have an important role in reducing the incidence of heart disease, lowering the percentage of fats in the blood, and reducing the oxidation of protein lipids through their work as antioxidant compounds and inhibitors of free radicals [6].Because of the importance of these compounds in human health, plant protection, and increasing oat yield, it has become necessary to pay attention to increasing quality characteristics of the grains. Given all of the above, this research aims to: Knowing the effect of the variety on the growth and productivity of oat grains. Also, measuring the effect of the variety on the grains content of flavonoids and their oxidative activity.

2-Materials And Methods

A field experiment was carried out in Babylon Governorate, Al-Shumali district, during winter season of 2022-2023 to evaluate the oxidative activity and grains content of some flavonoids compounds for four varieties of oat (Shifaa, Oats11, Algouda, and Carlup). The experiment was carried out in a randomized complete blocks design (RCBD) with three replications. The results were analyzed according to the ANOVA table and compared means according to the least significant differences test (LSD) at the probability level of 0.05. Random samples were also taken from the soil surface before plowing at 0-30 cm depth, mixed and dried to measure the physical and chemical properties of soil (Table 1).

Table 1: Chemical and physical properties of the study soil at a depth of 0–30 cm before oat planting.

Properties		**Values**
PH		**7.5**
EC		**1.14 ds/m^{-1}**
OM		**%1.6**
N available		**63.16 mg. kg^{-1}**
P available		**8.64 mg. kg^{-1}**
K available		**158 mg. kg^{-1}**
Soil separators	Sand	**%23**
	Silt	**%42**
	Clay	**%35**

The experimental soil was plowed, leveled, and divided into experimental units with dimensions (2 x 3m, each experimental unit containing 20 lines, the distance between the lines was 15 cm. Phosphate fertilizer was added in the form of triple superphosphate (P2O5 46%) at a rate of 80 kg ha^{-1} before planting. Nitrogen fertilizer was added at a rate of 120 kg N ha^{-1} in the form of urea fertilizer (46% N) in three equal batches: at the completion of emergence, the branching stage, and the flowering stage [7].The seeds

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were planted at a rate of 100 kg ha^{-1} on 11/17/2022. After collecting the data, it was analyzed using the statistical program GenStat V.20 according to the method approved by [8].The following characteristics were measured:

When the plants were 100% bloomed, random samples of ten plants were taken from the middle of each experimental unit.

1- Height of the main stem (cm): Each plant in the sample was measured with a tape measuring from the top of the soil to the bottom of the inflorescence on the main stem.

2- Area of the flag leaf (cm^2): It was calculated according to the equation approved by [9] as follows:

Flag leaf area = flag leaf length × maximum width × correction factor (0.75)

3- Number of tillers (tillers m^2): To determine the number of tillers per square meter, a random sample was selected from the center of each experimental unit by tossing a wooden frame of size 1m.

4- Number of f panicles (panicles m^2): The same approach used to determine the number of tillers was used to determine the total number of panicles at the peak of blooming..

5- Weight of 1000 grains (g): 1000 grains were taken randomly from the yield of each experimental unit, weighed, and returned to the yield.

6- Grains yield (ton ha^{-1}): A square meter was harvested from each experimental unit using the wooden frame. The grains were separated from the harvested sample plants, weighed, and then attributed to ton ha^{-1}.

7- Total flavonoids (mg kg^{-1} dry weight):

Oat grains extract was prepared according to the method used previously [10], by taking 3 g of ground grain powder for each treatment and placing it in 150 ml beaker and adding 60 ml of ethanol (C_2H_5OH), 95% concentration. The beakers were placed in a shaking water bath for a period 1.5 hour later, the extract was filtered using No.1 filter paper, after which the volume of the ethanol solvent was reduced using a rotary evaporator device at a temperature of 60°C until the volume of the samples was reduced to 10 ml. Then, the extracts were placed in marked test tubes and stored in the refrigerator at a temperature 4 ˚C to estimate some active compounds, and the method [11] was adopted to estimate the total flavonoids content of oat grains.

8 - Total antioxidant capacity (mg g^{-1} dry weight)

Total antioxidant capacity (TAC) was determined by estimating the reductive capacity of the grain extract for trivalent iron [12].

9- Effectiveness of free radical suppression (%)

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The method used by [13] was followed. A solution of the reagent 1,1-Diphenyl-2-picrylhydrazyl ($C_{18}H_{12}N_5O_6$) was prepared at a concentration of 0.1 mmol by taking 39.4 mg of the reagent and adding 100 ml of methanol (CH_3OH) with continuous stirring and after dissolving. Completely, other amounts of methanol were gradually added while simultaneously taking a reading of the optical absorption of the detector at the wavelength of 515 nm, which should be ± 0.1 to 0.7 nm. Then, 1 ml of extract from each sample was taken and 1 ml of the reagent was added. The tubes were mixed well and then left in dark place for four minutes at room temperature, after which the optical absorption was measured at a wavelength of 515 nm using a spectrophotometer. The optical absorption of the empty tube model was also recorded, then the percentage of oxidative activity in the grains was calculated by applying the following equation:

Oxidative activity = [(Optical absorption of the blank -Optical absorption of plant sample) / (Optical absorption of the blank sample)] × 100

10 - Flavonoid compounds in grains (mg kg^{-1} dry weight):

A sample was taken from grain mixing in each treatment with three replicates. The content of flavonoid compounds, which included Rutin, Catechin, Myricetin, Apigenin, Quercetin, and Kaempferol, was estimated in the laboratories of the Ministry of Science and Technology / Department of Environment and Water. The process of extracting samples was carried out according [14]. 0.5 g of grain samples was ground, then soaked with 10 ml of ethanol, 60% concentration. The samples were incubated in an ultrasonic bath for two hours, then the samples were centrifuged for 10 minutes at a speed of 7000 rpm, and the samples were filtered using filter paper with a transmittance of 0.45 micrometers. Getting rid of the ethanol using vacuum drying to make the final volume 1 ml. The separation and detection process was carried out according to [15] and modified according to [16] using a German-made High Performance Liquid Chromatography (HPLC) device, model (HPLC Sykam) with specifications shown in Table (2)

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Table 2. Specifications of the high-performance liquid chromatographic separation device

	Model	Component
1	S 1122	High pressure dual graduated pump
2	DAD S3240	Diode detector
3	S 5200	Sample loop (100 µl) and injector
4	Clarity	System analysis and control programs

The compounds were separated using C18-ODS separation column (length 250 mm, diameter 4.6 mm, particle size 5 micrometers, pore size 180 angstroms), German, The mobile phase consisted of the first solvent A (Acetonitrile 95% + 0.01% Trifluoroacetic) and the solvent The second B (Acetonitrile %5 + Trifluoroacetic %0.01), the flow rate was set to 1 ml min^{-1}, the temperature of the column was controlled thermostatically at 30 °C and the sample injection volume was 100 µl , and the separation time was as in Table (3).

Table 3 : Time to separate compounds with a high-performance liquid chromatographic separation device

	Solvent mixing ratios	Time
1	%10A %90 + B	0 – 5 min
2	%25A %75 + B	5 – 7 min
3	%40A %60 + B	7 – 13 min
4	%90A %10 + B	13 min - Until the next sample

Colorimetric detection was carried out using UV-Visible detector at wavelength of 278 nm, and the readings of the plant samples were calibrated according to the standard compounds for each of the flavonoids Quercetin, Myricetin, Kaempferol, Rutin, Catechin, and Apigenin at a concentration of 10 mg L^{-1}, according to the concentration of each compound according to the following equation:

Concentration of the substance (mg L^{-1}) = [Concentration of the standard compound (mg L^{-1}) x Area of the sample x Dilution factor] / [Area of the standard compound x Weight of the sample (mg)]

3-Results And Discussion

3-1 **vegetative growth**

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The results of Table 4. showed a significant effect of the variety on the characteristics of vegetative growth, as the Algouda variety was significantly superior in the characteristics of plant height and Flag leaf area, and gave the highest averages of 127.0 cm and 27 cm^2, respectively, compared to the Oat 11 variety, which gave the lowest averages of 94.2 cm. 24.4 cm^2, respectively. The Shifaa variety was also significantly superior in the number of tillers and recorded the highest average of 478.1 tillers m^{-2}, compared to the Algouda variety, which gave the lowest average of 425.8 tillers m^{-2}. The reason for the superiority of the Algouda variety in the height of the main stem may be due to the difference in genetic nature and the extent of its interaction and response to environmental factors. The reason for the increase in the flag leaf area of the Algouda variety plants may be attributed to the difference between the varieties in the amount of heat accumulation and light requirements to reach the flowering stage. As for the reason for the superiority of the Shifaa variety in number the tillers per square meter may be attributed to the difference in genetic nature that determines the amount of gene expression for longitudinal and transverse growth hormones [17]. These results agreed with [18, 19, 20, 21].

3-2 Traits of yield

The results of Table 4. showed that there was a significant effect of the variety on the yield characteristics, as the Shifaa variety was significantly superior in the characteristics of number of panicles, weight of 1000 grains, and grains yield (457.4 panicles m^{-2}, 36.97 g, and 7.15 tons ha^{-1} respectively). , compared to the oat variety 11, which gave the lowest averages of 403.2 panicles m^{-2}, 30.07 g, and 4.62 tons ha^{-1}, respectively. Perhaps the reason for the superiority of the Shifaa variety in the number of panicles are the ability of the variety to produce the highest number of tillers (Table 4), which led to an increase in the process of photosynthesis and production of dry matter, and thus this was reflected positively in the number of panicles. The reason for the superiority of the Shifaa variety in weight of 1000 grains is due to the difference in the genetic nature between the varieties in the storage capacity of the grains for dry matter, which led to the difference in their weights. The reason for the superiority of the Shifaa variety in grains yield is due to its superiority over the rest of the varieties in the number of tillers that produced panicles with grains and a weight of 1000 grains, which are considered among the basic components of the yield. The results are consistent with [21, 22, 23].

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Table 4. The effect of oat varieties on some characteristics of vegetative growth and yield

Cultivars	plant height)cm(	Flag sheet area (cm^2)	Number of tillers in m^2 (tillers m^2)	Number of spikes in m^2 (spike m^2)	Weight 1000 grain	grain yield (tha^{-1})
Shifaa	124.6	25.12	478.1	457.4	36.97	7.15
Oat11	94.2	24.4	437.1	403.2	30.07	4.62
Al-Algouda	127.0	27.0	425.8	410.6	33.59	6.26
Carlup	121.3	26.32	447.1	410.1	33.77	6.26
$L.S.D_{0.05}$	3.0	0.86	8.9	6.8	0.6	0.19

3-3 Oxidative activity in grains

The results of Table 5. showed a significant effect of the variety on the oxidative activity of grains. The Algouda variety was significantly superior in the characteristics of grains content of total flavonoids, free radicals inhibition efficacy and capacity of total antioxidant(163.8 mg kg^{-1} dry weight, 72.58%. 83.80 mg). The reason for the superiority of the Algouda variety in the grains content of total flavonoids may be attributed to the genetic nature of the variety in the extent of production of these compounds, as the amount of these compounds varies between plants according to the strength of the plant in expressing gene expression with the stages of plant development in its life cycle [24], or because Its superiority in plant height and thus increased biomass, which indicates an increase in the production of primary metabolic compounds through photosynthesis and thus an increase in the production of secondary metabolites accompanying the production of primary metabolites . This result is consistent with the findings of [25, 26].

Table 5. The effect of oat varieties on oxidative activity

Cultivars	Total flavonoids (mg kg^{-1} dry weight)	capacity of total antioxidant (mg g^{-1} dry weight)	free radicals inhibition efficacy (%)
Shifaa	160.62	73.42	70.97
Oat11	163.8	83.8	72.58
Al-Algouda	158	73.67	70
Carlup	158.02	80.45	71.04
$L.S.D_{0.05}$	2.3	1.07	1.33

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3-4 Grain content of flavonoids

The results of Table 6. showed a significant effect of the variety on the grains content of flavonoid compounds. The Algouda variety was significantly superior in the characteristics of the grains content of the compounds Quercetin, Myricetin, Kaempferol, Rutin, Catechin, and Apigenin, and gave the highest averages of 25.14, 13.23, and 19.98. 35.25, 14.38, 12.08 mg kg^{-1} dry weight, respectively, compared to the Oat11 variety, which recorded the lowest averages of 22.72, 11.28, 17.15, 32.19, 13.15, 9.76 mg kg^{-1} dry weight, respectively. The reason for the significant effect of the variety may be due to the content of the grains of the flavonoid compound Kaempferol is due to the genetic nature of the variety in the ratio of balance between phenolic and polyphenolic compounds. The reason for the superiority of the Algouda variety in the grains content of Quercetin may be attributed to its superiority in the vegetative growth of the plant, especially the height of the plant and the area of the flag leaf, as this compound of polyphenols accompanies auxins and maintains their activity in division and elongation [27]. This result is consistent with the findings of [25, 26, 28].

Table 6. The effect of oat varieties on the grain's content of some flavonoids (mg kg-1 dry weight)

Cultivars	Quercetin	Myricetin	Kaempferol	Rutin	Catechin	Apigenin
Shifaa	24.91	12.42	19.07	34.61	13.99	10.16
Oat11	22.72	11.28	17.15	32.19	13.15	9.76
lAlgouda	25.14	13.23	19.98	35.25	14.38	12.08
Carlup	23.30	12.65	18.61	34.69	13.20	11.32
P value ≤0.05	0.001					

Conclusion

It is concluded from the experiment that there was a significant effect of the variety on growth and yield characteristics, as the Algouda variety was significantly superior in most of the characteristics of vegetative growth, oxidative activity, total flavonoids, and flavonoid compounds in grains, while the Shifaa variety was superior in yield and its components.

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