



Effect of Some Aqueous Plant Extracts on Wheatgrass Growth Under Salinity Stress and Resulted Biscuits Property



Mohamed A Abd El-Azim¹, Usama A El- Behairy², Noura M Taha², Mamdouh MF Abdallah², Dina A Anwar^{1*}

Regional Center for Food and Feed, Agricultural Research Center, 12511, Giza, Egypt
 2- Horticulture Dept, Fac of Agric, Ain Shames Univ, P.O. Box 68, Hadayek Shubra 11241, Cairo, Egypt

*Corresponding author: <u>dinaanwar351@yahoo.com</u>

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Abstract: This study was conducted to examine the effects of the foliar application of moringa and germinated fenugreek extracts on the growth and biochemical parameters of wheatgrass grown under saline conditions. Aqueous moringa extract (MLE) was used at concentrations of 1.25, 0.83, 0.62 and 0.31%, whereas fenugreek extract (GFE) was used at concentrations of 0.25, 0.20, 0.15 and 0.10%. Optimum germination conditions were investigated under normal and NaCl stress (2000 ppm) conditions. Exposure to saline stress significantly reduced seed germination and grass yield as well as shoot and seedling fresh and dry weights. Ideal germination conditions were MLE 0.31% and GFE0.15%, resulting in increased growth traits compared with the other concentrations and control. Foliar application of MLE or GFE improved growth traits, total antioxidants, phenols, Ca, Mg, K, Mn, Cu, Zn and Fe in the presence or absence of NaCl. In addition, wheatgrass powder was used to replace wheat flour in developing biscuits at 5, 10 and 15% levels. Wheatgrass showed high feasibility for formulating nutrient-rich biscuits with acceptable sensory qualities. These findings provide useful information for enhancing the nutrients in biscuits, with potential use as a natural source of bioactive compounds in functional food products.

1 Introduction

Wheatgrass is the young grass of a common wheat plant, *Triticum aestivum* that is freshly juiced or dried into powder for consumption by both humans and animals. Wheatgrass is rich in antioxidants, vitamins (A, B, C, E and K), minerals and amino acids (Anwar et al 2015, Saleh et al 2021). Usually, the germination process under the conditions of arid regions requires the use of well water where the salinity reaches approximately 2000 ppm. During seed germination and growing seedlings, levels of various secondary metabolites have been affected by abiotic stress including salinity stress (Mencin et al 2021). Osmotic and ionic effects that are imposed by salinity have negative impacts on the important physiological and biochemical processes, which inhabit normal growth (Iqbal et al 2015). The application of plant extracts promote plant growth at a low cost and environmentally friendly environment; this stimulating action is concentration dependent (Abdel-Latef et al 2017). Moringa (Moringa oleifera) leaves are considered vegetables and belong to the Moringnance family. Its leaves contain high amounts of vitamins, minerals, phenolics and plant growth regulators e.g. zeatin and cytokinin (Rady and Mohamed 2015). Extracts of moringa leaves exhibit potential antioxidant properties due to their secondary metabolites. Whether in normal or stress conditions, treating numerous crops with moringa leaf extract (MLE) increased seed sprouting and seedling development. The extract increased the antioxidative, osmoprotective and ion uptake potentials of NaCl-stressed seedlings (Yasmeen et al 2013).

Fenugreek (Trigonella foenumgraecum L.) is a medicinal plant and belongs to the family Leguminosae commonly used in human nutrition and as an animal fodder for its abundance in phytochemical substances. Up to 1.0 % (w/v)of fenugreek seed extract efficiently improved the growth of maize and faba bean seedlings (Madany and Khalil 2017). However, germination induces the activation of many enzymes that augment the content of phytochemical components and the antioxidant capacity of the seeds (Ohanenye et al 2020). Most research was performed on the dry seed fenugreek extracts but little was done on the germinated state. Recently, functional foods have gained much attention. To maximize the health benefits, foods should be readily available in a form that makes them simple to consume (Pranil et al 2020). Recent studies cared about exchanging lifestyles and dietary habits which could be more helpful formodulating many health problems. Aljutaily et al (2022) reported that consuming biscuits supplemented with 10% date fiber decreased total cholesterol and triglyceride. In addition, many scientific studies showed that the supplementation of biscuits with dry moringa leaves improved their nutritional and physical properties (Hedhili et al 2021). In addition, Jiang et al (2022) showed that supplemented biscuits with papaya seeds and peels from 2 to 10% significantly increased the nutritional components and antioxidant properties. However, no information is recorded on the development of biscuits based on the levels of wheatgrass powder as a natural source of many nutrients. Accordingly, we aimed in this study to (1) investigate using aqueous extracts of moringa leaves and germinated fenugreek seeds to enhance the growth parameters and biochemical compounds of wheatgrass grown under salinitystress and (2) assess the possibility of using wheatgrassto formulate a nutrient-rich biscuits product.

2 Materials and Methods

2.1 Materials

Wheat grains (*Triticum aestivum*) and fenugreek seeds (*Trigonella foenumgraecum L.*) were obtained from the Field Crops Institute, Agricultural Research Center, Egypt. Young moringa leaves (*Moringa oleifera*) were collected from local farms in Ismailia, Egypt. Leaves were washed with tap water and dried in an oven at 55°C for 72 h. Dried leaves were powdered and sieved through a 40 mesh (420 μ m) sieve.

2.2 Preparation stock of moringa leaf extract (MLE)

Firstly, fresh moringa leaves were harvested and airdried then ground into fine powder. Moringa powder (100 g) was soaked in one liter of distilled water in the dark at room temperature for 24 hours with continuous stirring on the shaker and then filtered using What-man No.2 filter paper according to Sarmin (2014). Extracts were then frozen until used.

2.3 Preparation stock of germinated fenugreek extract (GFE)

Dry fenugreek seeds were soaked in water for 9 hrs, then drained and left to germinate for three days. Fenugreek sprouts were dried in an oven at 55°C for 72h and ground then sieved through a 40 mesh (420 μ m) sieve. For extraction of the fenugreek sprout's active ingredients, each 100g of fenugreek powder was soaked in 1L of distilled water for 24 hours with shaking. The mixture was then filtered using Whatman No.2 filter paper. Extracts were stored at -4 °C until used.

2.4 Wheatgrass growth and treatment condition

Wheat grains (116 g) were submerged in tap water for 12 hrs, drained and then regularly spread on the top of sterilized rice straw (as a growing medium). Water was applied as needed until grass was ready to harvest (about 8 days after seed sowing). At harvest, the greenwheatgrass was cut from a tray with scissors on the media surface.

2.4.1 Extract applications

Moringa leaf extract was diluted to four concentrations i.e. 1.25, 0.83, 0.62 and 0.31% while germinated fenugreek extract was diluted to 0.25, 0.20, 0.15 and 0.1%. Wheat grains were hydrated manually by spraying either moringa leaves or fenugreek seed extract besides the tap water control treatment. Germination was performed in triplicate.

2.4.2 Applications under salinity condition

From the previous experiment, the ideal concentrate of each extract was used in those experiments. Four experimental treatments were conducted according to the kind of extract as follows: (1) tap water: wheat grains were soaked and sprinkled with tap water, (2) saline water: grains were soaked and sprinkled with saline water at 2000 ppm, (3) tap water with extract: grains were soaked in tap water then sprinkled with moringa or germinated fenugreek extract and (4) saline water then sprinkled with moringa or germinated fenugreek extract: grains were soaked in saline water then sprinkled with moringa or germinated fenugreek extract.

2.4.3 Grass sampling

After 8 days of sprout (under indirect sunlight conditions), the grasses were collected to determine the following parameters: shoot length, weights of fresh and dry shoots and seedlings, fresh and dry grass yield per square meter, fresh and dry yield kg/kg of seeds and germination percentage.

2.5 Development of nutrient-rich biscuits

2.5.1 Preparation and experimental design for biscuit development

Wheatgrass was used to develop the traditional biscuits. To obtain wheatgrass flour, harvested grass was dried and ground into fine powder. Biscuits were prepared by replacing wheat flour with 5, 10 and 15% of grass flour. From previous experiments four kinds of grass flours were used, normal wheatgrass (WS), wheatgrass treated with moringa extract (MWS), wheatgrass treated with saline

water (SWS) and wheatgrass treated with fenugreek extract (FWS). Refined wheat flour (RF) was used for the preparation of control biscuits including the following ingredients: 56% flour, 21% shorting, 21% sugar, 1% baking powder, 0.5% salt and 0.5% vanilla. Ingredients were mixed, 10 min resting of dough, sheeting, laminating and cutting into a round shape. Biscuitswere baked in an electric oven at 180° C for min and cooled at room temperature.

2.5.2 Physical parameters of biscuits

Biscuits were tested for specific volume, thickness, diameter, and spread ratio. The specific volume of the biscuit was determined by measuring the biscuit volume (cm³) divided by its weight (g). The spread ratio was obtained by dividing the average diameter (W) by the average thickness (T) in triplicates (Bose and Shams-Ud-Din 2010).

2.5.3 Organoleptic evaluation

Twenty members of faculty staff and researchers from the Regional Center for Food and Feed were asked to score the main sensory characteristics (taste, odor, color and texture) of biscuits samples. Before starting the evaluation, all panelists were informed of all the experimental details. Evaluation of the biscuits was conducted 24 hours after baking. Biscuits were evaluated and degreed using a nine-point hedonic scale where 1 ="dislike extremely" and 9 = "like extremely"(Kortei et al 2020). Each sample was coded and the positions of the samples were randomized. Panelists wereseated in individual sensory booths and given distilled water to neutralize their mouths between the samples.

2.6 Biochemical analysis

2.6.1 Determination of total phenols (TP)

Total phenolic compounds in the different samples were analyzed by using Folin-Ciocalteu method (Singleton et al 1999). Gallic acid was used as standard and thedata were expressed as milligram gallic acid equivalentper 100 milliliters (mg GAE/L mL).

2.6.2 Determination of total antioxidants

Total antioxidant capacity was determined using the phosphomolebdenum method as reported by Prieto et al (1999). Results were expressed as milligram ascorbic acid equivalent per 100 milliliters (mg AAE/L mL).

2.6.3 Proximate composition

Protein, fat, fiber, and ash contents were determined according to methods described in AOAC (2016) while total carbohydrates were calculated according to Egan et al (1981). The moisture content of samples was measured according to the AOAC (2012).

2.6.4 Determination of minerals

Potassium, magnesium, phosphorous, calcium, manganese, copper, zinc, iron and sodium were analyzed according to the method described in the AOAC (2016).

2.6.5 Determination of chlorophyll content

The analytical determinations of chlorophyll a and b contents were performed according to Costache et al (2012). The absorbance of plant extracts was measured at thewavelengths of 666 and 653 nm and the calculationwas performed using the following equations:

Chlorophyll A = 15.65 A666- 7.340 A653 Chlorophyll B = 27.05 A653 - 11.21 A666

2.7 Statistical analysis

Data were analyzed using complete randomized ANOVA according to Gomez and Gomez (1984). Duncan's test was conducted using the CostateStatistics program V.6.4 (USA).

3 Results and Discussion

3.1 Effect of moringa extracts on wheatgrass growth

Moringa leaf extract was analyzed and its chemical composition was as follows: phosphorus 0.254 ppm, calcium 2039 ppm, magnesium 668 ppm, potassium 2346 ppm, manganese 10.25 ppm, cupper 4.61 ppm, zinc 3.55 ppm, ferric 14.72 ppm, sodium 9.32 ppm, total antioxidant 1303.9 ppm and total phenols 1240.1 ppm. **Table 1** shows that shoot and seedling fresh and dry weights were significantly increased by treating with 0.31% moringa extract compared with other concentrations and tap water. MLE 0.31% produced a maximum fresh grass yield per square meter (1.745) andper kilogram of seeds (1.558), whereas MLE 1.25% produced a minimum yield; a similar trend was noticed

for dry shoot yield. These findings were supported by Cheema et al (2012) who testified that a very tiny amount of foliar spraying of moringa water extract had some noticeable benefitsand improved yield. Application of MLE at 0.31% enhanced germination percentage, which was also statistically at par with the application of 0.62% and tap water (control). Moringa leaves are rich and good sources of antioxidants, potassium, calcium, and micro-nutrients that promote plant growth and are used as exogenous plant growth enhancers (Abdalla 2013 and Iq-bal et al 2020). Besides, Iqbal et al (2020) reported that the growth of wheat plants can be enhanced by the application of moringa extract which regards wheat hormones that boost the different stages of plant growth and development. The rapid increase in growth can also e attributed to the enriched content of crude proteins; this concept follows those reported by Moyo et al (2011) who studied the growth-promoting hormones and proteins present in moringa leaves and observed their responsibility, individually or jointly, in the gradual and rapid increase in crop growth. Zeatin, which canbe considered the common form of naturally occurringcytokinin in plants, has been found in high concentrations in moringa leaves.

3.2 Effect of moringa extract, saline water and their interaction on the growth and yield of wheatgrass

The effects of salinity and moringa extract on the growth parameters of wheatgrass were investigated. No significant differences were observed in the results of salinity treatment compared with control (tap water). However, NaCl application (2000 ppm) significantly decreased dry shoot yield (Table 2). The effect of moringa extract (0.31%) treatment on the growth of wheatgrass was conspicuous which recorded an increment in mean shoot length and fresh weight of shoot, seedling and grass yield by 3.81%, 10.77%, 17.79% and 12.26%, respectively over non-extract-applied seedlings. In addition, the foliar application of moringa extractenhanced the growth of wheatgrass grown under salinity and the values were significantly higher than those plants in the presence of salinity alone. Using MLE for plants enhanced the activities of antioxidant enzymes and levels of carotenoids and proline either in the absence or presence of NaCl. This observation could be due to that moring a extract is rich in some antioxidants (particularly ascorbic acid), proline and phytohormones especially (cytokinins). Treating plants with aqueous MLE significantly ameliorated the ionic and osmotic deteriorations resulting from Na uptake by seedlings, in addition to sustaining ionic and osmotic homeostasis in Na-stressed seedlings (Howladar 2014). MLE induces the endogenous hormonal level causing improved plant

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Moringa concentrations	1 259/	0.920/	0.629/	0 210/	Control
Characters	1.23 /0	0.03 /0	0.02 /0	0.3170	(tap water)
Shoot length (cm)	12.56 ^a	13.58 ^a	13.67 ^a	14.08 ^a	13.89 ^a
Shoot fresh weight (mg)	70.03 ^b	75.48 ^b	78.86 ^b	91.87ª	74.39 ^b
Seedling fresh weight (mg)	148.2 ^b	153.3 ^b	170.2 ^{ab}	191.6 ^a	154.8 ^b
Shoots fresh weight (kg/m ²)	1.396°	1.430 ^c	1.576 ^b	1.745 ^a	1.586 ^b
Shoots fresh weight (kg/kg of seeds)	1.246 ^c	1.276 ^c	1.406 ^b	1.558 ^a	1.416 ^b
Shoot dry weight (mg)	6.00 ^b	6.74 ^b	7.52 ^{ab}	8.91ª	7.37 ^b
Seedling dry weight (mg)	12.71 ^b	13.70 ^b	15.60 ^{ab}	18.28 ^a	14.90 ^b
Shoots dry weight (kg/ m ²)	119.7°	127.8°	147.3 ^b	169.4 ^a	146.3 ^b
Shoots dry weight (kg/kg of seeds)	106.9°	114.0 ^c	133.1 ^b	151.2 ^a	130.6 ^b
% Germination	72.23 ^b	73.02 ^b	87.90 ^a	89.72ª	88.14 ^a

Table 1. Effect of using different concentrations of moringa leaf extract on the growth characteristics of wheatgrass

Mean values in each row having different superscripts ($a, b, c \dots$) are significantly different at p < 0.05 according toDuncan's test.

Table 2. Effect of using moringa leaf extract (MLE), saline water and their interactions on the growth characteristics of wheatgrass

Charac	cters	Shoot length	Shoot fresh	Seedling fresh	Shoots fresh	Shoots fresh weight	Shoots dry weight (kg/kg of	Shoots dry weight	Seedling dry weight	Shoot dry weight
Treatm	ents	(cm)	weight (mg)	weight (mg)	weight (kg/m²)	(kg/kg of seeds)	seeds)	(kg/m ²)	(mg)	(mg)
Tap water	Without MLE	12.84 ab	77.77 ^b	165.5 bc	1.531 ab	1.366 ab	129.6 bc	145.2 bc	15.69 ^b	7.37 bc
	With MLE	13.29 ª	86.50 ª	195.6 ª	1.672 ª	1.493 ª	150.8 ª	168.9 ª	19.76 ª	8.73 ª
Mea	n	13.07 ª	82.14 ª	180.5 a	1.601 ª	1.430 a	140.2 ª	157.1 ª	17.73 a	8.05 ª
Saline water	Without MLE	12.29 ^b	73.37 b	157.1 °	1.373 b	1.226 b	117.0 °	131.0 °	14.97 ^b	6.99 °
3	With MLE	12.82 ab	80.92 ^{ab}	184.4 ab	1.588 ª	1.418 a	139.1 ab	155.8 ab	18.38 ª	7.93 ab
Mea	n	12.56 ª	80.92 ^{ab}	170.7 ª	1.480 ª	1.322 ª	128.1 ^b	143.4 ^b	16.68 ª	7.46 ª
Mean	Without MLE	12.57 b	75.57 b	161.3 b	1.452 ^b	1.296 b	123.3 b	138.1 ^b	15.33 b	7.18 ъ
Private (SP2) (18335)	With MLE	13.05ª	83.71 ª	190.0ª	1.630 ª	1.456 a	145.0 ª	162.4 ª	19.07 ª	8.33 ª

Mean values in each column having different superscripts (a, b, c \dots) are significantly different at p< 0.05 according toDuncan's test.

growth in the presence and/or absence of any other stressful factor. To sum up, the present study proved MLE as a potent eco-friendly approach to relieve the harmful effects of sodium ions.

3.3 Effect of germinated fenugreek extract on the growth parameters of wheatgrass

Germinated fenugreek extract was analyzed and its chemical constituents were as follows: phosphorus 0.487 ppm, calcium 811 ppm, magnesium 457 ppm, potassium 6033 ppm, manganese 10.0 ppm, cupper 7.11 ppm, zinc 25.15 ppm, ferric 105.2 ppm, sodium 21.2 ppm, total antioxidant 1048.2 ppm and total phenols 406.6 ppm. Data in **Table 3** revealed that growth parameters were affected significantly by foliar application with germinatedfenugreek extract (GFE). Maximum shoot and seedling fresh and dry weights were recorded for 0.15% and 0.10% of GFE in comparison with other treatments. Increasing the concentration of fenugreek extract severely retarded the growth of wheatgrass. However foliar application up to 0.15% of GFE im- proved significantly the fresh and dry yield of wheat-grass. Similarly, the 0.15% fenugreek treatment significantly increased the germination percentage of wheat seeds by about 13.8% compared to control. These results are in accordance with those reported by Madanyand Khalil (2017) who reported that

Treatments					
Characters	0.25%	0.20%	0.15%	0.10%	Tap water
					(control)
Shoot length (cm)	12.18 ^a	12.81ª	13.13 ^a	13.46 ^a	12.98ª
Shoot fresh weight (mg)	65.96 ^c	80.82 ^b	100.1ª	101.6ª	82.72 ^b
Seedling fresh weight (mg)	169.2 ^b	177.3 ^b	209.0ª	212.5ª	178.6 ^b
Shoots fresh weight (kg/m ²	1.497°	1.542 ^{bc}	1.808 ^a	1.652 ^b	1.603 ^{bc}
Shoots fresh weight (kg/kg of seeds)	1.337°	1.377 ^{bc}	1.614 ^a	1.476 ^b	1.432 ^b
Shoot dry weight (mg)	6.03°	7.54 ^b	9.66ª	9.57ª	7.74 ^b
Seedling dry weight (mg)	15.50 ^b	16.56 ^b	20.19 ^a	20.03ª	16.72 ^b
Shoots dry weight (kg/ m ²	137.1°	144.0 ^{bc}	174.6 ^a	155.7 ^b	150.2 ^b
Shoots dry weight (kg/kg of seeds)	122.4°	128.6 ^{bc}	155.9ª	139.1 ^b	134.1 ^b
% Germination	74.15°	79.19 ^b	93.29ª	88.71 ^b	79.49 ^b

 Table 3. Effect of using different concentrations of germinated fenugreek extract on the growth characteristics of wheatgrass

Mean values in each row having different superscripts (a, b, c \dots) are significantly different at p< 0.05 according to Duncan's test.

treated maize seedlings with lower concentrations of fenugreek seed extract (0.25, 0.50, and 1.0%; w/v) improved all assessed growth criteria which suggests that these treatments hadno negative effects; however, the highest level of fenugreek extract declined all the measured growth parameters. They also analyzed fenugreek seed extract and indicated the presence of coumarin, p-hydroxybenzoic acid, salicylic acid, syringic acid, vanillic acid, caffeic acid, ferulic acid, 4-hydroxycinnamic acid and sinapic acid. They claimed that the combined actions of these compounds could contribute to the observed plant growth elevation or delay dependingon the used extract concentration. Debski et al (2021) found also p-hydroxybenzoic acid as the predominant phenolic acid of fenugreek sprouts while germination of seeds can liberate insolublebound phenolics. Accordingly, it can be recommended to use GFE at low concentrations with a preference of 0.15%.

3.4 Effect of germinated fenugreek extract, saline water and their combination on the growth parameters of wheatgrass

In the current study, wheat seedlings treated with NaCl exhibited a reduction in the growth parameters as shown in **Table 4**. Compared with the control, Na stress resulted in a significant reduction in the fresh and dry weights of both shoot and yield. Foliar application of aqueous fenugreek extract (GFE) was considerably effective in increasing the growth parameters of non-stressed wheat seedlings, in addition to significantly alleviating the growth reduction mediated by Na. GFE contains 1048.2 ppm of total antioxidants and 406.6 ppm of total phenols. Since the accumulation of phenolics and increasing activity of antioxidants help the plant to acclimatize in stress conditions; fenugreek extract can enhance seedling's growth under saline conditions.

3.5 Effect of MLE and GFE on the biochemicalanalysis of wheatgrass under salinity condition

3.5.1 Effect on protein

Application of MLE alone or in combination with salt elevated the content of protein over those of either the untreated control or NaCl-stressed wheat shoots (Fig 1). On the other side, an opposite pattern was noticed in protein content after treated wheat seedlings with GFE, where salt stress re- resulted in a dramatic accumulation of protein content with the highest accumulation (28.5%) at NaClstress, compared to GFE and control. These resultsmay be due to the potential protein content in the plant extracts in addition to the possibility of the accumulation of the proline amino acid under saline stress. According to the study by Ghezal et al (2016), many plants can store proline as a defense osmolyte when they are under saline stress. Additionally, some suitable solutes, such as proline, can maintain the activity of the enzymes found in saline solutions.

Char Soaki	racters ng seeds	Shoot length (cm)	Shoot fresh weight (mg)	Seedling fresh weight (mg)	Shoots fresh weight (kg/m²)	Shoots fresh weight (kg/kg of seeds)	Shoot dry weight (mg)	Seedling dry weight (mg)	Shoots dry weight (kg/m²)	Shoots dry weight (kg/kg of seeds)	% Germination
Tap	Without GFE	11.87 ª	88.41 ^b	184.8 ^b	1.681 ^b	1.501 ^b	7.67 ^b	16.06 ^b	145.8 ^b	130.2 ^b	86.30 ^b
water	With GFE	12.31 ª	106.3 ª	203.9 ª	1.765 ª	1.576 ª	10.04 ª	19.26 ª	166.7 ª	148.9 ª	91.82 ª
М	Iean	12.09 a	97.37 a	194.4 ª	1.723 a	1.539 ª	8.86 ^a	17.66 ª	156.3 ª	139.6 ª	89.06 ª
Saline	Without GFE	11.54 a	77.41 °	160.4 °	1.505 °	1.344 °	7.07 ^b	14.66 °	137.5 °	122.8 °	77.33 °
water	With GFE	11.79 ª	87.35 b	176.8 ^b	1.640 ^b	1.465 ^b	7.97 ^b	16.13 b	149.6 ^b	133.6 ^b	84.02 ^b
М	lean	11.67 ª	82.38 b	168.6 ^b	1.573 b	1.405 b	7.52 b	15.39 b	143.6 ^b	128.2 ^b	80.67 ^b
Mean	Without GFE	11.71 ª	82.91 ^b	172.6 ^b	1.593 b	1.423 b	7.37 b	15.36 b	141.7 ^b	126.5 b	81.81 ^b
	With GFE	12.05 ª	96.84 ª	190.3 ª	1.703 ª	1.520 ª	9.00 ª	17.69 ª	158.2 ª	141.2 ª	87.92 ª

Table 4. Effect of using germinated fenugreek extract (GFE), saline water and their interactions on the growth characteristics of wheatgrass

Mean values in each row having different superscripts (a, b, c ...) are significantly different at p < 0.05 according to Duncan's test



Fig 1. Protein content of wheatgrass using (a) moringa leaf extract (MLE) and (b) germinated fenugreek extract (GFE) under saline stress

3.5.2 Effect on antioxidant activity and total phenols

Total antioxidants and phenols exhibited an increased trend in response to both MLE and GFE treatment (**Fig 2**). The presence of NaCl alone or in association with extracts influenced the accumulation of antioxidant activity and phenols in wheatgrass. A similar observation was reported by Howladar (2014) whoshowed that plants treated with moringa extract caused a significant increase in the antioxidant enzymes. Besides, exposing plants to NaCl alone increased the levelof the antioxidant enzymes. In addition, Gomes and Garcia (2013) found that TPC was elevated during seed germination, particularly under stress conditions. Antioxidants including total phenolics are developed in cells in response to the oxidative stress resulting from the production and accumulation of ROS in seeds. The impacts of TPC accumulationin sprouts under stress conditions have been also shownin mung beans (Moongngarm et al 2021) and chickpeas (León-López et al 2020).



Fig 2. Phytochemicals content (ppm) of wheatgrass using (a) moringa leaf extract (MLE) and (b) germinated fenugreek extract (GFE) under saline stress

3.5.3 Effect on minerals

In normal conditions, wheat grass contained 3780 ppm of P, 443 ppm of Ca, 457 ppm of Mg, 4380 ppm of K, 34.98 ppm of Mn, 1.66 ppm of Cu, 18.97 ppm ofZn, 52.68 ppm of Fe and 32.18 ppm of Na (Fig 3). Upon salt stress, the concentration of Na was increased in the wheat shoots accompanied by an increase in the content of P, Ca, Mn, Zn and Fe. However, Mg and K were decreased by 48.57% and 14.16%, respectively incomparison with the control (tap water). Exogenous fo-liar application of MLE and GFE improved the uptake of Ca, Mg, K, Cu and Zn and decreased the content of Na under both normal and saline conditions. Accordingto Rehman et al (2021), the exogenous application of MLE enhanced wheat development by maintaining thehigh moisture content in tissues through the accumula- tion of K and Ca. Moringa leaves are abundant in sub- stances that promote growth and contain Ca, K, and Fe, which are considered growth supporters (Gopalakrishnan et al 2016).

3.5.4 Effect on photosynthetic pigments

The application of Na was extremely destructive concerning photosynthetic pigments as observed in **Fig 4**. The shoots content of chlorophyll a and b significantly declined with percentages of 15.5 and 9.58 %, respectively compared to the control. Furthermore, chlorophyll a was reported to bemore sensitive to lead stress than chlorophyll b. Foliar application with MLE or GFE triggered an enhanced response and ameliorated Na-stress by raising the level of chlorophyll contents as compared to NaCl treatment. These results agreed with those of Yasmeen et al (2013) who reported that MLE improved the total chlorophyll contents of wheat plants subjected to salt stress. Moreover, Latif and Mohamed (2016) reported that the foliarspray of plants with MLE significantly improved the chlorophyll content in salt-stressed plants. Theyattributed this effect to the high content of macro-nutrients in extracts especially magnesium, which is a main constituent of chlorophyll.

3.6 Enriched biscuits with wheatgrass powder

3.6.1 Sensory characteristics of biscuits

Biscuits prepared from different flour blends were evaluated for taste, color, odor, and texture by sensory evaluation (Table 5). Generally, different recipes did not significantly affect all sensory parameters. However, grass flour level had variable effects on the acceptability of biscuits. Biscuits recipes containing 5% of wheatgrass flour have high scores on the taste while increasing the substitutionlevel to 15% caused a rejection of biscuits (scores less than 5). Color measurement is very important in determining the acceptability of food products (Kohajdová et al 2013). As the levels of grass flourget higher (10 and 15%), the color of supplemented biscuits gets progressively darker. It was also foundthat higher levels of grass flour also hurt the odor of the final products due to the higher intensity of dry grass odor. The addition of grass flour increased slightly the hardness of the biscuits especially when using 15% of wheatgrass flour. The hardnessof biscuits has been changed by the addition of various seed flours (Kohajdová et al 2013). According to Oyeyinka and Bassey (2023), the increase in hardness could be related to disruption in the well-defined protein-starch complex of the dough which is related to the reduction in the wheat structure forming starch and protein.



Fig 3. Minerals content (ppm) of wheatgrass using (A) moringa leaf extract (MLE) and (B) germinated fenugreek extract (GFE) under saline stress



Fig 4. Chlorophyll contents (mg/100g) of wheatgrass using (a) moringa leaf extract (MLE) and (b) germinated fenugreek extract (GFE) under saline stress

3.6.2 Physical characteristics

Data in **Table 6** revealed that specific volume was significantly higher in the MWS and FWS treatments. On the other hand, the volume increased with the low- ering level of grass flour in biscuits. Kohajdová et al (2013) explained that "the decreasing in volume is mostlikely due to the combined effect of the disruption of gluten network structure and the dilution of gluten by the flour particles". The diameter of the biscuits in- increased in SWS and FWS treatments with low inclusion of grass flour in the biscuit's formation. The thickness and spread ratio was not significantly affected either bythe kind of flour or the level of grass flour in the product. Both spread ratio and diameter are usually used to assess the quality of flour used in biscuit preparation and the ability of the biscuit to rise (Bala et al 2015), where a higher spread ratio of biscuit is preferred (Chauhan et al 2016).

Treatm	ents	Taste	Color	Odor	Texture
	5%	8.60 ^a	8.90 ^{ab}	8.90 ^{ab}	8.70 ^a
	10%	6.70 °	7.00 ^f	8.70 ^{ab}	8.60 ^a
$\mathbf{RF} + \mathbf{WS}$	15%	4.50 ^d	7.60 def	8.50 ^b	8.60 ^a
Mea	n	6.60 ^a	7.83 ^b	8.70 ^a	8.63 ^a
	5%	9.00 ^a	9.00 ^a	9.00 ^a	9.00 ^a
	10%	6.70 °	7.80 ^{c-f}	8.40 ^b	8.50 ^a
RF + MWS	15%	4.70 ^d	7.40 ef	8.40 ^b	8.50 ^a
Mea	Mean		8.06 ^b	8.60 ^a	8.66 ^a
	5%	8.70 ^a	8.60 abc	8.70 ^{ab}	9.00 ^a
RF + SWS	10%	6.60 °	8.40 ^{a-d}	8.60 ^b	8.90 ^a
M 15005	15%	4.70 ^d	8.00 ^{b-e}	8.60 ^b	8.80 ^a
Mea	n	6.66 ^a	8.33 ^{ab}	8.63 ^a	8.90 ^a
	5%	8.80 ^a	8.70 abc	8.80 ^{ab}	8.80 ^a
RF + FWS	10%	7.30 ^b	8.60 abc	8.70 ^{ab}	8.70 ^a
MI I I WB	15%	4.60 ^d	8.50 ^{a-d}	8.60 ^b	8.60 ^a
Mean		6.90 ^a	8.63 ^a	8.70 ^a	8.70 ^a
	5%	8.77 ^a	8.80 ^a	8.85 ª	8.87 ^a
Mean	10%	6.82 ^b	7.97 ^b	8.60 ^b	8.67 ^{ab}
	15%	4.62 °	7.87 ^b	8.52 b	8.62 ^b

Table 5. Sensory evaluation of biscuits

RF= refined flour, WS= wheat grass, MWS= wheat grass treated with moringa extract, SWS= wheat grass under saline conditions, FWS= wheat grass treated with germinated fenugreek extract. Mean values in each column having different superscripts (a, b, c ...) are significantly different at p< 0.05 according to Duncan's test

Table 6. Physical properties of wheatgrass flour incorporated biscuits

Treatments		Specific volume	Diameter (mm)	Thickness (mm)	Spread ratio
	5%	1.18 ^a	63.6 ^a	2.30 bc	3.6 ^b
$\mathbf{RF} + \mathbf{WS}$	10%	0.97 ^a	64.3 ^{ab}	2.20 ^{cd}	3.4 ^{cd}
	15%	0.93ª	63.6 ^{ab}	2.30 bc	3.6 ^b
Mea	n	1.03 ^a	63.8 ^b	2.27 ^a	3.5 ^a
	5%	0.93 ^a	64.0 ^{ab}	2.30 bc	3.6 ^b
RF + MWS	10%	0.95 ^a	63.3 ^b	2.40 ^{ab}	3.8 ^a
	15%	0.95ª	64.0 ^{ab}	2.10 ^d	3.2 ^{ef}
Mean		0.94 ^b	63.8 ^b	2.27ª	3.5 ^a
	5%	0.95ª	66.0 ^{ab}	2.20 ^{cd}	3.3 ^{cde}
RF + SWS	10%	0.94 ^a	64.6 ^{ab}	2.50 ^a	3.8 ^a
	15%	0.95 ^a	66.3 ^{ab}	2.30 bc	3.4 ^{cd}
Mea	n	0.95 ^b	65.6 ^a	2.33ª	3.5 ^a
	5%	0.97ª	65.6 ^{ab}	2.30 bc	3.5 ^{bc}
RF +FWS	10%	1.06 ^a	66.0 ^{ab}	2.10 ^d	3.1 ^f
	15%	0.96ª	65.0 ^{ab}	2.50 ^a	3.8 ^a
Mean		1.00 ^a	65.5 ^a	2.30 ^a	3.5 ^a
	5%	1.01 ^a	64.8ª	2.28ª	3.5ª
Mean	10%	0.98 ^{ab}	64.6 ^a	2.30 ^a	3.6ª
	15%	0.95 ^b	64.8 ^a	2.30 ^a	3.5ª

RF= refined flour, WS= wheat grass, MWS= wheat grass treated with moringa extract, SWS= wheat grass under saline conditions, FWS= wheat grass treated with germinated fenugreek extract. Mean values in each column having different superscripts (a, b, c ...) are significantly different at p< 0.05 according to Duncan's test.

3.6.3 Proximate chemical analysis of biscuits

Proximate analysis of biscuits shown in **Table 7** indicated that protein content was increased from 8.40% (control sample) to 9.80% (sample with 5% FWS). Proteins have a crucial role in growing, mending and maintaining children's bodies. In addition, proteins serve ascarriers for other nutrients such as lipids, vitamin A, iron, sodium, and potassium (Mahan and Escott-Stump 2008). Enriched biscuits with wheat grass flour causedan increase in fiber and ash contents. This increase is more favorable since it is known that fiber aids the digestive system in humans. Conversely, the fat and carbohydrate contents of biscuits decreased with the use ofwheatgrass flour.

3.6.4 Mineral contents of biscuits

Table 8 presents the mineral composition of biscuit samples; the Ca and Mg contents of sample 3 (RE + MWS) were higher than in other biscuit samples. The increase percentages were 119.9 and 221.2 % respectively over control. Calcium helps blood clotting and creates strong healthy bones and teeth (Theobald 2005). Magnesium is a cofactor in more than 300 enzyme systems that control a variety of biochemical processes in the body, including protein synthesis, blood glucose control, blood pressure regulation and muscle and nerve function. Mg maintains a regular heartbeat and strong bones (Al Alawi et al 2018). Table 8 also reveals that sample 5 (RF + FWS) was higher in K, Mn, Cu and Zn than other treatments. It is worth noting that Na content increased as more wheatgrass flour was incorporated into biscuits.

Treatments	Protein	Fiber	Lipids	Carbohydrates	Moisture	Ash
100% RF	8.40 ^b	0.28 ^d	20.40 ^a	68.71ª	2.20 ^c	0.01 ^e
RF + 5% WS	9.50 ^a	0.74 ^c	19.64 ^b	65.44°	3.52 ^a	1.16 ^d
RF + 5% MWS	9.70 ^a	1.22ª	19.06 ^e	66.12 ^{bc}	2.14 ^d	1.76 ^a
RF + 5% SWS	9.60 ^a	1.13 ^b	19.22 ^d	65.71 ^{bc}	2.73 ^b	1.53 ^b
RF + 5% FWS	9.80 ^a	1.21ª	19.38°	66.40 ^b	1.85 ^e	1.44 ^c

Table 7. Proximate analysis (g/100g) of biscuits

RF= refined flour, WS= wheatgrass, MWS= wheat grass treated with moringa extract, SWS= wheat grass under saline conditions, FWS= wheat grass treated with germinated fenugreek extract. Mean values in each column having different superscripts (a, b, c ...) are significantly different at p< 0.05 according to Duncan's test.

Treatments	100% RF	RF+WS	RF+MWS	RF+SWS	RF+FWS
Minerals (ppm)					
Phosphorus (P)	20.37°	27.88 ^{bc}	40.21 ^{ab}	35.66 ^{ab}	43.24 ^a
Calcium (Ca)	100.3 ^e	162.6 ^d	220.6 ^a	184.8 ^c	190.6 ^b
Magnesium (Mg)	41.65 ^e	90.65°	133.8ª	54.25 ^d	101.2 ^b
Potassium (K)	205.1 ^e	275.3°	285.3 ^b	254.3 ^d	308.5ª
Manganese (Mn)	0.798 ^d	0.800 ^d	1.446 ^b	1.078 ^c	1.881 ^a
Copper (Cu)	1.876 ^e	2.674 ^c	2.825 ^b	2.081 ^d	3.059 ^a
Zinc (Zn)	0.714 ^e	0.987 ^d	1.440 ^b	1.124 ^c	1.554 ^a
Ferric (Fe)	1.000e	1.654 ^d	2.014 ^b	1.877°	2.247ª
Sodium (Na)	44.20 ^e	55.64 ^d	67.24 ^b	71.24ª	59.35°

Table 8. Mineral contents of biscuits

RF= refined flour, WS= wheat grass, MWS= wheat grass treated with moringa extract, SWS= wheat grass under saline conditions, FWS= wheat grass treated with germinated fenugreek extract. Mean values in each row having different superscripts (a, b, c ...) are significantly different at p< 0.05 according to Duncan's test

4 Conclusion

Wheatgrass is a young grass of wheat plant that can be juiced or dried into powder for human consumption. Growing grass under salt stress lowered its growth characteristics. However, foliar application with moringa or germinated fenugreekextracts improved and increased the seedling growth both in the presence or absence of NaCl. Extracts with low concentrations (0.31% for moringaextract and 0.15% for fenugreek extract) were more effective than the higher ones in enhancing the germination percentage and grass yield. Moreover, the application of extracts improved different biochemical constituents in grasses under saline conditions. Wheatgrass is promising as a good source of nutrients to develop nutrient-rich biscuits. The incorporation of wheatgrass flour increased the content of protein and minerals in biscuits as compared with the control sample which can be used as functional ingredients in food manufacturing.

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References

Abdalla MM (2013) The potential of *Moringa oleifera* extract as a biostimulant in enhancing the growth, biochemical and hormonal contents in rocket (*Eru cavesicaria* subsp. sativa) plants. *International Journal of Plant Physiology and Biochemistry* 5, 42–49.

https://doi.org/10.5897/IJPPB2012.026

Abdel -Latef AA, Abu Alhmad M, Ahmad S, et al (2017) Foliar application of fresh moringa leaf extract overcomes salt stress in fenugreek (*Trigonella foenum-graecum*) plants. *Egyptian Journal of Botany* 57, 157-179. https://ejbo.journals.ekb.eg/article 2630.html

Al Alawi AM, Majoni SW, Falhammar H (2018) Magnesium and human health: perspectives and research directions. *International Journal of Endocrinology* 2018, 9041694.

https://doi.org/10.1155/2018/9041694

Aljutaily T, Elbeltagy A, Ali AA, et al (2022) Anti-obesity effects of formulated biscuits supplemented with date's fiber; agro-waste products used as a potent functional food. *Nutrients* 14, 5315. https://doi.org/10.3390/nu14245315

Anwar DA, Abou El-Yazied A, Mohammadi TF, et al (2015) Wheatgrass juice and its nutritional value as affected by sprouting condition. *Arab Universities Journal of Agricultural Sciences* 23, 37-49. https://doi.org/10.21608/ajs.2015.14558

AOAC (2012) Official Methods of Analysis. Association of Official Analytical Chemists, 19th ed; Gaithersburg, MD, USA.

AOAC (2016) Official Methods of Analysis of AOAC International. 20th ed Latimer GW (Ed). Arlington: AOAC International.

Bala A, Gul K, Riar CS (2015) Functional and sensory properties of cookies prepared from wheat floursupplemented with cassava and water chestnut flours.*Cogent Food and Agriculture* 1, 1019815. https://doi.org/10.1080/23311932.2015.1019815

Bose D, Shams-Ud-Din M (2010) The effect of chickpea (Cicer arietinim) husk on the properties of cracker biscuits. *Journal of the Bangladesh Agricultural University* 8, 147–152.

http://dx.doi.org/10.22004/ag.econ.208478

Chauhan A, Saxena DC, Singh S (2016) Physical, textural and sensory characteristics of wheat and amaranth flour blend cookies. *Cogent Food and Agriculture* 2, 1125773.

https://doi.org/10.1080/23311932.2015.1125773

Cheema ZA, Farooq M, Khaliq A (2012) Application of Allelopathy in Crop Production: Success Story fromPakistan. In: Cheema ZA, Farooq M, Abdul Wahid,(Eds), Allelopathy: Current Trends and FutureApplications, Springer, Berlin, Heidelberg Germany pp 113-143. <u>https://doi.org/10.1007/978-3-642-30595-5_6</u>

Costache MA, Campeanu G, Neata G (2012) Studies concerning the extraction of chlorophyll and total carotenoids from vegetables. *Romanian Biotechnological Letters* 17, 7702-7708. <u>http://surl.li/ighvjx</u>

Debski H, Wiczkowski W, Szawara-Nowak D, et al (2021) Elicitation with sodium silicate and iron chelate affects the contents of phenolic compounds and minerals in buckwheat sprouts. *Polish Journal of Food Nutrition Sciences* 71, 21–28.

https://doi.org/10.31883/pjfns/131061.

Egan HI, Kirk RS, Sawyar R, et al (1981) Pearson'sChemical Analysis of Food. (8th ed), Churchill Livingstone, 591 Pp. <u>http://surl.li/ppjrsm</u>

Ghezal N, Rinez I, Sbai H, et al (2016) Improvement of *Pisum sativum* salt stress tolerance by biopriming their seeds using *Typha angustifolia* leaves aqueous extract. *South African Journal of Botany* 105, 240 – 250.

https://doi.org/10.1016/j.sajb.2016.04.006

Gomes MP, Garcia, QS (2013) Reactive oxygen species and seed germination. *Biologia* 68, 351–357. <u>https://doi.org/10.2478/s11756-013-0161-y</u>

Gomez KA, Gomez AA (1984) Statistical Producer for Agricultural Research 2nd Ed., John Wiley and Sons Inc., Singapore, 691 Pp. https://worldveg.tind.io/record/3801/

Gopalakrishnan L, Doriya K, Kumar DS (2016) *Moringa oleifera*: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness* 5, 49-56. https://doi.org/10.1016/j.fshw.2016.04.001

Hedhili A, Lubbers S, Bou-Maroun E, et al (2021) *Moringa oleifera* supplemented biscuits: Nutritional values and consumer segmentation. *South African Journal of Botany* 138, 406-414. https://doi.org/10.1016/j.sajb.2021.01.017

Howladar SM (2014) A novel *Maringa oleifera* leaf extract can mitigate the stress effects of salinity and cadmium in bean (*Phaseolus vulgaris* L.) plants. *Ecotoxicology and Environmental Safety* 100, 69-75.

https://doi.org/10.1016/j.ecoenv.2013.11.022

Iqbal MA, Cheema ZA, Afzal MI (2015) Evaluation of forage soybean (*Glycine max* L.) germination and seedling growth enhancement by seed priming techniques. *American-Eurasian Journal of Agricultural and Environmental Sciences* 15, 1198–1203. http://surl.li/jvxsoq

Iqbal J, Irshad J, Bashir, S, et al (2020) Comparative study of water extracts of moringa leaves and roots to improve the growth and yield of sunflower. *South African Journal of Botany* 129, 221-224. https://doi.org/10.1016/j.sajb.2019.06.032

Jiang G, Feng X, Zhao C, et al (2022) Development ofbiscuits supplemented with papaya seed and peel: Effects on physicochemical properties, bioactive compounds, in vitro absorption capacities and starch digestibility. *Journal of Food Science and Technology* 59, 1341-1352.

https://doi.org/10.1007%2Fs13197-021-05143-z

Kohajdová Z, Karovičová J, Magala M (2013) Rheological and qualitative characteristics of pea flour incorporated cracker biscuits. *Croatian Journal of Food Science and Technology* 5, 11–17. https://hrcak.srce.hr/106157

Kortei NK, Odamtten GT, Obodai M, et al (2020) Sensory evaluation, descriptive textural analysis, and consumer acceptance profile of steamed gamma-irradiated *Pleurotus ostreatus* (Ex.Fr.) Kummer kept in two different storage packs. *Scientific African* 8, e00328. https://doi.org/10.1016/j.sciaf.2020.e00328

Latif HH, Mohamed HI (2016) Exogenous applications of moringa leaf extract effect on retrotransposon, ultrastructural and biochemical contents of common bean plants under environmental stresses. *South African Journal of Botany* 106, 221-231. https://doi.org/10.1016/j.sajb.2016.07.010

León-López L, Escobar-Zúñiga Y, Salazar-Salas NY, et al (2020) Improving polyphenolic compounds: Antioxidant activity in chickpea sprouts through elici-tation with hydrogen peroxide. *Foods* 9, 1791. https://doi.org/10.3390/foods9121791

Madany M Khalil RR (2017) Fenugreek seed extract enhanced the growth of *vicia faba* and *zea mays*seedlings. *Egyptian Journal of Botany* 57, 363–377. http://dx.doi.org/10.21608/ejbo.2017.777.1047

Mahan LK, Escott-Stump S (2008) Krouse's Food and Nutrition Therapy.12th ed, Saunders Elsevier, Canada, 1352 p. <u>https://cmc.marmot.org/Record/.b32718342</u>

Meena M, Divyanshu K, Kumar S, et al (2019) Regulation of L-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. *Heliyon* 5, e02952. <u>https://doi.org/10.1016%2Fj.heliyon.2019.e02952</u>

Mencin M, Abramovič H, Jamnik P, et al (2021) Abiotic stress combinations improve the phenolics profiles and activities of extractable and bound antioxidants from germinated spelt (*Triticum spelta* L.) seeds. *Food Chemistry* 344, 28704.

https://doi.org/10.1016/j.foodchem.2020.128704

Moongngarm A, Chaiyarak A, Prachanun K, et al (2021) Improved melatonin content and antioxidant activity of mung bean sprout by germinationunder salinity incorporated with exogenous melatonin. 7th International Conference on Food, Agriculture and Biotechnology, (29-30 July 2020), Thailand, *Journal of Sustainability science and Management* 16, 79-93. Moyo B, Masika PJ, Hugo A, et al (2011) Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. *African Journal of Biotechnology* 10, 12925-12933.

https://doi.org/10.5897/AJB10.1599.

Ohanenye IC, Tsopmo A, Ejike CE, et al (2020) Germination as a bioprocess for enhancing the quality and nutritional prospects of Legume proteins. *Trends in Food Science and Technology* 101, 213-222. <u>https://doi.org/10.1016/j.tifs.2020.05.003</u>

Oyeyinka SA, Bassey IAV(2023) Composition, functionality and baking quality of flour from four brands of wheat flour. *Journal of Culinary Science and Technology* 1-21.

https://doi.org/10.1080/15428052.2023.2191874

Pranil T, Moongngarm A, Loypimai P (2020) Influence of pH, temperature, and light on the stability of melatonin in aqueous solutions and fruit juices. *Heliyon* 6, e03648.

https://doi.org/10.1016/j.heliyon.2020.e03648

Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry* 269, 337– 341. <u>https://doi.org/10.1006/abio.1999.4019</u>

Rady MM, Mohamed GF (2015) Modulation of salt stress effects on the growth, physio-chemical attributes and yields of *Phaseolus vulgaris* L.

plants by the combined application of salicylic acid andmoringa oleifera leaf extract. *Scienta Horticulturae*193, 105–113.

https://doi.org/10.1016/j.scienta.2015.07.003

Rehman H, Alharby HF, Bamagoos AA, et al (2021) Sequenced application of glutathione as an antioxidant with an organic biostimulant improves physiological and metabolic adaptation to salinity in wheat. *Plant Physiology and Biochemistry* 158, 43–52.

https://doi.org/10.1016/j.plaphy.2020.11.041

Saleh EMM, El-Sahar EG, Elewa RS (2021) A study on the effect of wheatgrass on nutritional status and some blood parameters in rats with suppressed immune system. *African Journal of Biological Sciences* 17, 51– 61. https://doi.org/10.21608/ajbs.2021.153142

Sarmin NS (2014) Effect of *Moringa oleifera* on germi nation and growth of *Triticum aestivum*. *Journal of Bioscience and Agriculture Research* 2, 59-69. http://dx.doi.org/10.18801/jbar.020214.20

Singleton VL, Orthofer R, Lamuela- Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology* 299, 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

Theobald HE (2005) Dietary Calcium and Health. *Nutrition Bulletin* 30, 237-277.

https://doi.org/10.1111/j.1467-3010.2005.00514.x

Yasmeen A, Basra SMA, Farooq M, et al (2013)Exogenous application of moringa leaf extract modu-lates the antioxidant enzyme system to improve wheatperformance under saline conditions. *Plant GrowthRegulation* 69, 225-233.

http://dx.doi.org/10.1007/s10725-012-9764-5