Enhancement of nutraceutical and antioxidant potential of sunflower hybrid seed varieties through chemical priming

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Abstract: Pathogenic agents cause an increased risk of various fatal diseases and there is a need to reduce this risk using medicinal plants and their seeds. The present research work was designed to study the efficacy of different sunflower seed hybrid varieties (i.e. FH622, FH620, FH615, FH613 and FH545) chemically primed with potassium nitrate as natural antioxidant and antimicrobial agent. Antioxidant potential was determined using DPPH test, reducing power, TPC and TFC. Antibacterial activity was determined against Gram positive and Gram negative bacterial species. After one week, the germination data including mean germination and percentage of final emergence was calculated. It was found that seed varieties FH620 and FH615 have higher values of mean germination as compared to FH545 while FH615 has higher percentage of final emergence as compared to FH620 and FH545. High phenolic and flavonoid contents were observed in FH620 and FH615 as compared to FH545 variety. It was also observed that seed variety FH615 when treated with KNO_3 solution had significantly high germination as well as antioxidant parameters and antibacterial activity as compared to other varieties. Similarly FH615 showed high antibacterial activities against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria. This study showed that all selected sunflower hybrids have good antioxidant and antibacterial potentials that would further be used for different trials to cure different pathogen related diseases, and these are natural sources of antioxidants for commercial and therapeutic applications.

Keywords: Sunflower seeds varieties, germination parameters, total phenolic contents, total flavonoid contents.

INTRODUCTION

To improve pre-sowing seed germination different promising techniques have been used. Among these techniques seed priming is the most important one which helps to accelerate the germination process by increasing the metabolic rate of plants (Bukhari *et al*., 2019). Currently, health related issues are increasing greatly and there is a need to check factors responsible for different diseases. For this purpose, different medicinal plants and oil seeds play a significant role to cure and control health related issues due to the presence of active metabolic compounds e.g. antioxidants (phenolic, flavonoid and many other compounds) (Mustafa *et al*., 2017). These compounds are effective when taken in diet and also linked with decreased risks of fatal diseases (Sharif *et al*., 2017).

In recent years, oil seed crops have been emphasized for their positive effects on disease prevention and health improvement. Due to a significant contribution in health improvement, sunflower seeds have also received attention (Anjum *et al*., 2012). The sunflower plant (*Helianthus annuus* L.) is a leading oil seed crop that is mainly grown for its seeds and ranks second in the world after soybean oil for edible oil production (Stefansson, 2007). Due to presence of phenolic, flavonoid, biologically active compounds, polyunsaturated fatty acids and vitamins sunflower seeds contain valuable antioxidant, antimicrobial, anti-inflammatory, anti-hypertensive, wound healing and cardiovascular benefits (Sharif *et al*., 2018). Sunflower has been used to make oil, meal and many other products as it contains high linoleic acid contents. Due to high oleic acid concentrations the sunflower has wide spectrum of applications in pharmaceutical sciences. Sunflower seeds have high antibacterial properties that help to reduce use of antibiotics in treatment of many infectious diseases (Aboki *et al*., 2012).

Seeds of sunflower plants are treated with different priming techniques including chemical priming with potassium nitrate to overcome delayed seed germination (Garcia-Lopez *et al*. 2014). Studies have shown that growth hormones such as GA3, KNO_3, thiourea and sodium azide are helpful in breaking the dormancy of plants and their seeds (Khan and Shah 2011). It is well known that exogenous NO_3 ion application can increase

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the nitrate contents of salt-stressed plants and improve salinity tolerance to different levels (Jabeen and Ahmad, 2011). The aim of present study was therefore to investigate the biological and antioxidant activities of sunflower by chemical priming with potassium nitrate solution. As nitrate ions play a role in biological and antioxidant activities so it was hypothesized that potassium nitrate could enhance the germination rate, antioxidant and biological potentials of sunflower seeds remarkably.

MATERIALS AND METHOD

Taxonomically identified five hybrid seed varieties (i.e. FH622, FH620, FH615, FH613 and FH545) of sunflower (*Helianthus annuus* L.) were taken from Oil Seed Center, Ayub Agriculture Research Institute, Faisalabad, Pakistan. It was found that the seed varieties FH620, FH615 and FH545 indicated high percentage of growth (i.e. FH620>FH615>FH545). On the basis of these parameters, these three hybrid seed varieties were chosen for further analyses.

Seed priming with bioagents and germination parameters

Sunflower hybrid seeds (FH620, FH615 and FH545) were treated with 3% solution of KNO_3. Seeds were dipped in 3% KNO_3 solution (10-15mL) in Petri plates and incubated at room temperature for 12 h. After treatment, seeds were grown in Petri plates in a germination incubator at Department of Crop Physiology, University of Agriculture, Faisalabad. All the experiments were designed in triplicates and germination data was taken at 7^{th} day of sowing. On 7^{th} day of germination, final emergence percentage (FGP) was calculated by the formula given below (Ijaz *et al*., 2012).

$$FGP = \left(\frac{Ng}{Np}\right) \times 100$$

Where last number of emerged seeds is presented by Ng and total number of seeds is presented by Np.

Mean growth time (MGT) presented in days was calculated using the formula given as:

$$MGT = \frac{\sum(Dn)}{\sum n}$$

Total number of seeds is presented by n and day on which germination was started to test the germination time is presented by D.

Extract preparation

Dried sunflower seedlings were ground in distilled water at 10:1 (mg/mL) ratio. After filtration, the filtrate was used for antioxidant studies.

Antibacterial assay

Antibacterial activity of sunflower seeds extract was checked by agar well diffusion method using Gram positive (*S. aureus*) and Gram negative (*E. coli*) bacterial strains. Nutrient agar medium was autoclaved and poured in sterilized Petri plates and allowed to solidify at room temperature. Wells were made by sterilized cork borer and poured suitable quantity of test sample in these wells against standard drug ampicillin for antibacterial activity. Petri plates were incubated at 37ºC overnight. After that zones of inhibition were measured in millimeter by zone reader.

Minimum inhibitory concentration (MIC) determination

For determination of minimum inhibitory concentration, 100µL sterilized medium of nutrient broth was put in each well and mixed with 100µL of seed extracts of selected sunflower hybrid varieties by two fold serial dilution method. Then 10µL of tested bacterial strain culture was added and covered the 96-well plate by aluminium foil and incubated at room temperature for 24 h. Next day 10µL resazurin (270 mg tablet of resazurin in 40mL dist. water) was added in each well and observed the color changes from purple to light pink. Change in color indicates the minimum concentration of sample that is unable for the growth of bacteria and pink color indicates the growth of bacteria. Change in color at lowest concentration was taken as minimum inhibitory concentration (MIC).

Total phenolic contents (TPC)

Phenolic contents were determined using Folin Ciocalteu (FC reagent) that was prepared freshly. The sample extract (0.2mL) was mixed with 800µL of FC reagent along with 200µL of (7.5%) Na_2CO_3 solution. Mixture was incubated in dark for 2 h at room temperature and absorbance was taken at 765 nm. Gallic acid (GAE) was used as a standard and results were expressed in mg/g units (Ojo and Anibijuwon, 2010).

Total flavonoid contents (TFC)

Flavonoid contents were determined by taking 100 µL of sample extract which was mixed in 300 µL aluminum chloride (10%) solution, 300 µL of (5%) $NaNO_2$ solution, and 1 mL of distilled water. After 5 minutes, 200 µL of 1 mM NaOH solution was added. This reaction was kept for 10 min at room temperature and absorbance was taken at 510 nm. The results were expressed as mg/g. Quercetin (QE) was taken as a standard for flavonoid content estimation (Ojo and Anibijuwon, 2010).

1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay

Free radical scavenging assay was performed by dissolving the DPPH powder in methanol (0.004 mg/100 mL). The sample extract (0.05µL) was mixed with 5mL of DPPH solution and kept in dark for 30 min at room temperature. Absorbance was taken at 517 nm against blank (reaction mixture without sample extract) and percentage of inhibition was calculated by the formula given below. Ascorbic acid was taken as a standard (Feizi *et al*., 2012).

$$\%\ \text{scavenging}\ activity = \frac{\text{Control}_{Abs} - \text{Test sample}_{Abs}}{\text{Control}_{Abs}} \times 100$$

Reducing power ability

Reducing power assay was performed by taking 0.1 mL of sample extract with 5 mL of potassium phosphate (2 M, pH 6.6) and 5 mL of (1%) $Fe(CN)_6$ solution and incubated for 20 min at room temperature. Then trichloroacetic acid (TCA) (1%, 5 mL) was added and centrifuged at 3,000 rpm for 10 min. After centrifugation, the upper layer (5 mL) was mixed with 5 mL distilled water and 1 mL $FeCl_2$ (0.1%) solution and the absorbance was measured at 700 nm. Increased absorbance indicates increased power reduction (Hegazy and Ibrahim, 2012) that was calculated by formula given below:

$$\%\ \text{reducing}\ power = \frac{\text{Test sample}_{Abs} - \text{Control}_{Abs}}{\text{Control}_{Abs}} \times 100$$

Total antioxidant assay

For total antioxidant assay, 2 mL of reaction mixture consisting of 100 µL of H_2SO_4 (0.6 M), 4 mM of ammonium molybdate and 28 mM of Na_2PO_4 solution were added in 20 mL of distilled water and made the final volume to 50 mL that was mixed with 1 mL of sample extract. Reaction mixture was incubated for 60 min at 30°C. Absorbance was taken at 665 nm against blank (contained reagent mixture only). Percentage of total antioxidant assay was calculated by formula given below (Murthy *et al*., 2011).

$$\text{Total antioxidant}\ activity(\%) = \frac{\text{Control}_{Abs} - \text{Sample}_{Abs}}{\text{Control}_{Abs}} \times 100$$

STATISTICAL ANALYSIS

The statistical analysis was carried out to optimize different growth parameters. Data of all biochemical parameters was computed using GraphPad prism software (version 5) at a 95% confidence interval and $p<0.05$ was considered as significant.

RESULTS

Pre-treatment given to sunflower seeds with potassium nitrate has led to significant changes in physiological characteristics and plant growth. The seedlings growth parameters (percentage of mean germination and final germination) were measured.

Effect of potassium nitrate on seed growth

The analysis of variance showed significant ($p\leq0.05$) difference in percentage of germination due to major effect of KNO_3 concentration. The duration of priming and interaction of concentration of KNO_3 and duration of priming were presented in fig. 1.

Fig 1(A) showed the percentage of mean germination time. It was indicated that seed variety FH615, when treated with KNO_3 solution, revealed the highest value of percentage of mean germination time (17.00±0.12) as compared to other seed varieties.

Fig 1(B) is showing percentage of final emergence. It was indicated that seed variety FH615 when treated with KNO_3 solution showed the highest value of percentage of final emergence as compared to other seed varieties.

Total flavonoid and total phenolic contents

Phenolic and polyphenolic compounds directly contribute to antioxidant action and constitute as major category of naturally occurring antioxidants in plants (Awika *et al*., 2003). Consequently, it is necessary to evaluate total phenolic contents in different species of plants. TPCs were determined by method introduced by Follin Ciocalteu and the results were presented as equivalents of Gallic-acid.

The results given in fig 2(A) indicated that the total phenolic contents were found to be the highest in the sunflower hybrid variety FH620 while the lowest total phenolic contents were found in variety FH545. From the fig 2(B) it was indicated the sunflower hybrid variety FH615 when treated with 3% KNO_3 showed the highest contents of total flavonoids while the lowest contents of total flavonoids were exhibited by FH545.

DPPH free radical scavenging activity

Data presented in table 1 gives detailed information about the DPPH free radical scavenging activity. From the comparative analysis, it was found that seed variety FH545 had the highest DPPH scavenging activity as compared to other seed varieties.

Reducing Power Assay

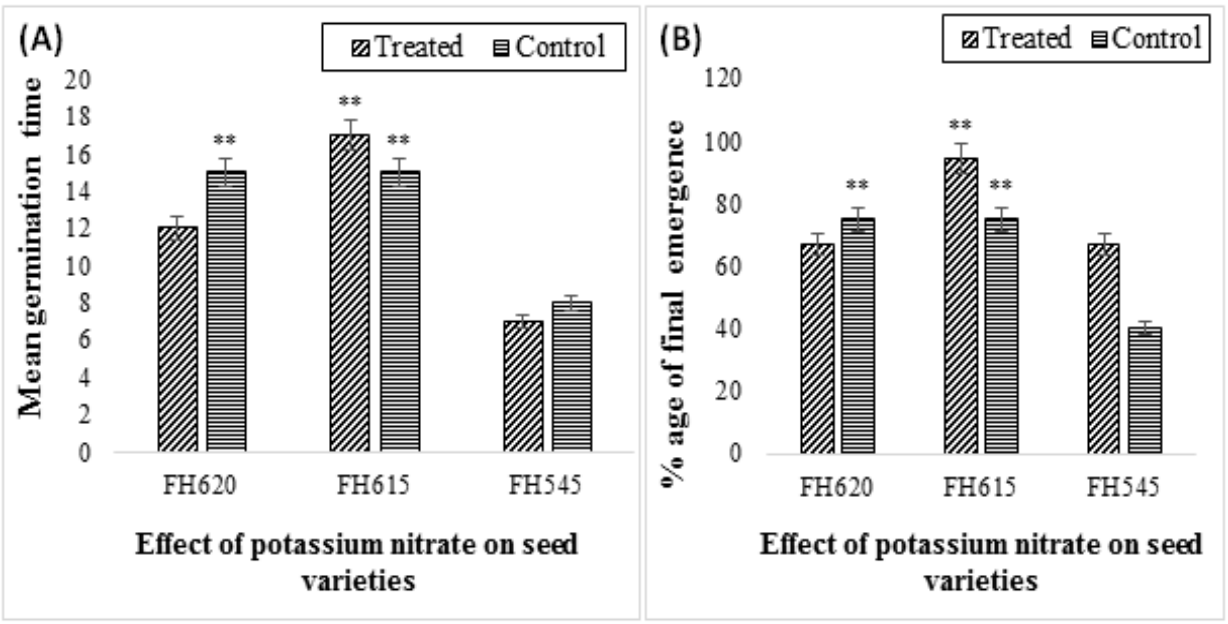
The results of reducing power assay have been given in table 1. It was revealed that seeds of FH615 variety treated with KNO_3 solution exhibited the highest reducing power activity than other selected varieties and FH620 showed the lowest reducing power activity.

Total antioxidant capacity

From table 1 it was found that total antioxidant capacity of FH615 was the highest than other seed varieties while that of FH545 was the lowest.

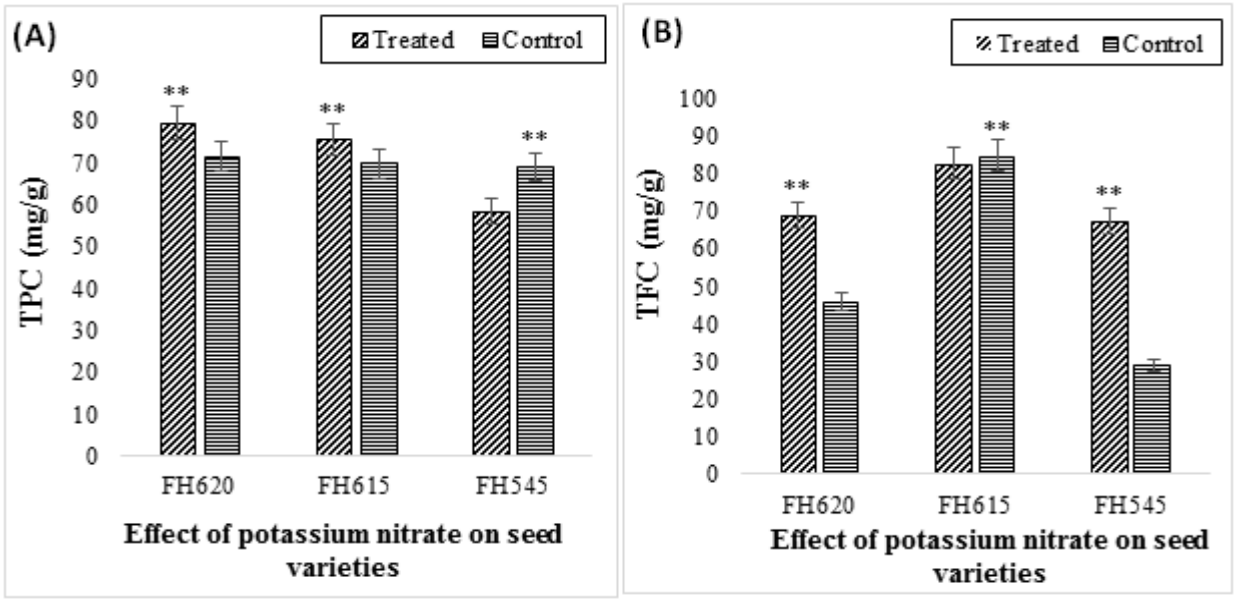
Antibacterial activity

Antibacterial activities of extracts of sunflower hybrid varieties were determined against one Gram positive (*S. aureus*) and one Gram negative (*E. coli*) bacterial strain (table 2). The extracts showed significant antimicrobial activities as compared to the standard antibiotic (i.e. ampicillin). FH620 seed extract exhibited large zone of inhibition (16±0.80) and low value of MIC (20±0.40) µg/mL against *E. coli* as compared to un-primed seed extracts while un-primed seed extract of FH545 exhibited low (7±0.21) zone of inhibition and large value of MIC (98±0.18) µg/mL.



Level of significance ($p \leq 0.05$) indicated by symbol '**'

Fig 1: Effect of Potassium nitrate on (A) mean germination time and (B) percentage of final emergence of Sunflower hybrid varieties



Level of significance ($p \leq 0.05$) indicated by symbol**'**'**

Fig 2: Effect of potassium nitrate on (A) total phenolic contents, (B) total flavonoid contents of Sunflower hybrid seed varieties

DISCUSSION

The results of this study have revealed that seed primed for 6-12 hours with KNO_3 have positive effects on percentage of seed germination. It may be owing to seed priming that helps in protrusion of testa or development of antioxidant potential during priming process. Halo-priming for 6-12 hours resulted in the highest percentage of sunflower seed germination (Selvarani and Umarani, 2011) that was correlated with the germination of FH615 hybrid variety of sunflower that showed high mean germination as well as final emergence percentage. In addition, Mekonnen (2005) noted that the germination rate was consistently reduced by extending the duration of halo-priming with KNO_3 to 72 hours. Too long priming onto the seed germination could possess negative effect.

Table 1: Antioxidant activities of sunflower hybrid seeds varieties

Seed Varieties	Treated with KNO_3	%age of DPPH	%age of Reducing power	%age of Total antioxidant capacity
FH620	Treated	40.06 ± 0.12^{c}	36.57 ± 0.78^{e}	61.53 ± 0.38^{b}
	Control	40.07 ± 0.15^{c}	45.78 ± 0.57^{d}	60.38 ± 0.53^{b}
FH615	Treated	52.8 ± 0.60^{b}	68.57 ± 0.45^{a}	70.76 ± 0.83^{a}
	Control	58.48 ± 0.51^{b}	58.84 ± 0.57^{c}	58.84 ± 0.76^{c}
FH545	Treated	55.8 ± 0.45^{b}	67.71 ± 0.67^{a}	48.07 ± 0.38^{c}
	Control	61.04 ± 0.15^{a}	64.85 ± 0.75^{b}	60.76 ± 0.94^{b}

Values are mean ± SD of 3 separate determinations. Different letters as superscripts are showing significant difference among extract of sunflower hybrid varieties.

Table 2: Antibacterial activity of sunflower hybrid verities against Gram positive and Gram negative bacterial strains

Seed varieties	Treated with KNO_3	Antibacterial activity			
		S. aureus		*E. coli*	
		Zone of inhibition (mm)	MIC (µg/mL)	Zone of inhibition (mm)	MIC (µg/mL)
FH620	Treated	14 ± 0.90^{c}	68 ± 0.88^{c}	16 ± 0.80^{b}	20 ± 0.40^{e}
	Control	12 ± 0.15^{d}	60 ± 0.18^{d}	14 ± 0.35^{d}	37 ± 0.18^{e}
FH615	Treated	18 ± 0.37^{b}	8 ± 0.22^{e}	15 ± 0.30^{c}	70 ± 0.56^{c}
	Control	14 ± 0.55^{c}	12 ± 0.58^{b}	12 ± 0.33^{e}	68 ± 0.98^{c}
FH545	Treated	12 ± 0.5^{d}	75 ± 0.80^{e}	10 ± 0.79^{f}	98 ± 0.18^{a}
	Control	9 ± 0.23^{e}	98 ± 0.52^{a}	7 ± 0.21^{g}	85 ± 0.20^{b}
Ampicillin	--	24 ± 0.38^{a}	2 ± 0.92^{f}	21 ± 0.11^{a}	5.8 ± 0.16^{f}

Values are mean ± SD of 3 separate determinations. Different letters as superscripts are showing significant difference among extract of sunflower hybrid varieties.

The variation in antibacterial activities of selected seed varieties against Gram positive *S. aureus* may be due to change in chemical composition of seed varieties when treated with KNO_3 as nitrates play a significant role as an antioxidant agent. Similarly, in case of Gram negative (*E. coli*) strain the seed extract of FH620 showed highest antibacterial activity among all selected seed varieties. This is because of the presence of high concentration of tocopherol and phenolic compounds. It was also previously reported that some compounds e.g. monoterpenes and their derivatives are responsible for antibacterial activities (Gupta *et al*., 2016). Comparative analysis indicated that sunflower hybrid variety FH615 showed batter results of phenolic contents as compared to other varieties. Fiska *et al*. (2006) determined total phenolic contents in the seeds of sunflower with a dry weight of 2700 mg/100 g. Our result are comparable with the results given by Weisz *et al*. (2009) by taking amounts of all contents individually which ranged from 90.54 mg/100g up to 15.95mg/100g dry-matter for seed hybrids. They also noticed the TPC of kernels of sunflower were found to be higher as compared to the results determined in the shells.

Comparatively stable DPPH radical has generally been used to test compounds with ability to act freely as hydrogen donors and radical scavengers to calculate the antioxidant properties of these compounds (Atta *et al*., 2017). DPPH is stable and commercially available organic nitrogen radicals associated with different *in vivo* oxidative reactions (Kuganesan *et al*., 2017). Free radical scavenging activity of DPPH is one of those indicators which are important in determining or extracting the antioxidant potentials of selected bioactive molecules. For extracts of these seed varieties, several studies using DPPH activity for the determination of free radical-scavenging activity of different oil-seeds of different plants such as sunflower had showed high antioxidant values (Fei *et al*., 2015). The Fe^{2+}, (transition metal ion) has great ability to shift single electron so that many radical reactions can be formed and propagated with relatively non-reactive radicals (Aboul-Enein *et al*., 2003). The most important approach for preventing the generation of reactive oxygen species with active redox metal catalysis is to chelate the metal ions. From comparative analysis, it was found that seed variety FH615 treated with KNO_3 had the highest percentage of reducing power as compared to other seed varieties while untreated seed variety FH545 has the highest percentage of reducing power. Matthaus *et al*. (2002) reported that the antioxidant properties of residues of sunflower are to be nearly 70%. Mezza *et al*. (2018) also found significant antioxidant activity of sunflower seeds. A positive linear-relationship between total phenolic acidic contents and antioxidant activity was observed in the varieties of sunflower seeds tested (Zilic *et al*., 2010).

KNO_3 indirectly provide source of nitrogen which plays a significant role in protein synthesis at genetic level. At

initial level of seedling rate protein synthesis is more as compared to late stage of plant development. Rajeswari *et al*. (2010) have reported that KNO_3 supplemented MS basal medium with a concentration of 1.9 gl^{-1} is suitable for the embryogenesis in cotton. They have also observed that in addition to the importance of NO^{3-} for the enhancement of cell growth and the percent embryo induction, the presence of K^+ also plays an important role in such processes. Moreover, KNO_3 is more helpful in the maturation of somatic embryo as compared to the induction of somatic embryo.

CONCLUSION

It is concluded that all the studied sunflower seed hybrid varieties possessed antioxidant activity potentials, high contents of total phenolics and flavonoids. Sunflower seed hybrid varieties including FH620 and FH615 showed high contents of total phenolics while FH615 had the highest total flavonoid contents. Untreated FH545 seed extract had the highest DPPH free radical scavenging activity while extract of FH615 had the highest percentage of total antioxidant capacity and reducing power in contrast to other seed varieties as well as untreated seeds. It was also revealed that seed variety FH615 showed strong antibacterial activity against Gram positive (*S. aureus*) and Gram negative (*E. coli*) bacteria and low values of MIC in case of *S. aureus* and high values of MIC for *E. coli*. This finding indicates that low concentration of sample extract is highly effective to inhibit bacterial growth which will help to improve the defensive system of sunflower seeds varieties when treated with potassium nitrate. It needs further studies to assess their potential components as effective natural remedies.

ACKNOWLEDGEMENT

The authors highly acknowledge the Oil Seed Center of Ayub Agriculture Research Institute, Faisalabad, Pakistan for providing hybrid varieties of sunflower seeds, and Department of Biochemistry, and Department of Crop Physiology, University of Agriculture Faisalabad, Pakistan for providing facilities and lab space.

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