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BIOCHEMICAL AND PHENOLIC ACID PROFILING OF SUNFLOWER HYBRID VARIETIES' SEED TREATED WITH DIFFERENT BIO-PRIMING AGENTS

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Abstract

Seed dormancy is a major factor in germination of seeds. Sunflower is the most important oil seed crop that faces a major problem in seed dormancy. Therefore, a study was conducted to find out the efficiency of biocontrol agents such as *Enterobacter* (FD-17), *Bacillus* sp. (KS-54) and *Paraburkholderia phytofirmans* (PsJN) on seed germination and biochemical profile of sunflower seedlings. These biocontrol agents have mineralization and solubilization mechanism of nutrients from organic and inorganic sources that reduce the chances of seed dormancy and help in germination of seeds. Growth parameters of seedlings (germination percentage, mean growth time, vigor index I, vigor II) and enzymatic parameters (catalase, peroxidase, superoxide dismutase, protease activity, total soluble protein and amylase activity) were measured. It was shown that the seed variety FH620 has (100%) germination percentage, (40%) mean growth time when treated with *Enterobacter* (FD-17) and *Paraburkholderia phytofirmans* (PsJN). Similarly, biochemical profiling indicated that FH620 gave optimized results when treated with *Enterobacter* (FD-17) strains. Similarly, in case of protease, lipid peroxidation (MDA) contents and α-amylase, the seed variety FH620 indicated 10.33 mg/mL of protease when treated with (*Bacillus* sp.) KS-54, (95.52 mg/mL) MDA contents when treated with (*Enterobacter)* FD-17. Phenolic profile was also carried out by high performance liquid chromatography. All the experiments were performed in triplicates under optimized conditions.

Key words: Enterobacter, Germination parameters, Biochemical profiling, HPLC, FH620.

Introduction

Pakistan is very rich in its plant biodiversity and has a tradition of using great number of medicinal plants to cure equally great number of diseases but without knowledge of bioactive constituents these plants have (Mustafa *et al*., 2016; Bukhari *et al*., 2021). The demand of herbal medicines and many natural products from different plant species has been consistently increasing for past many years (Mustafa *et al*., 2017; 2020). A great number of medicinal plants have been used to cure different diseases because of their therapeutic potentials governed by various phytochemical constituents such as alkaloids, glycosides, flavonoids, saponins, steroids and tannins (Bukhari *et al*., 2019; Munir *et al*., 2020). Sunflower (*Helianthus annuus* L.) is one of the most dynamic oilseed crops cultivated in the world (Zia ud Den *et al*., 2019). Besides soya bean, rapeseed and palm oil, sunflower oil is the 4th important vegetable oil with annual production of about 9 million tones in world trade. It is also an amusing source of edible and oil seed crop that is suitable to agro-climatic circumstances (Kaleem *et al.,* 2011). Due to dormancy, huge amount of seeds become waste or do not grow properly or if grow then such type seeds give very poor germination results at seedling stage. Sometime seeds have very poor mechanism of mineralization and solubilization of nutrients that are present in soil due to this they do not grow properly. Therefore, it is recommended that seed priming is mostly the cause of rapid growth which has practical agronomic position in crop production, particularly under adverse environmental conditions (Mc Donald, 2000).

Different priming techniques have been widely used to solve the problems related to germination under different environmental conditions and among them biopriming is an important technique for application of microorganisms to seeds (Zaheer *et al.,* 2012). Bio-priming is a method in which soil borne diseases are handled using biocontrol mediators with the help of priming (El-Mougy, 2007; Mohamedy, 2013). In biopriming seeds are primed with different endophytic microbes that easily integrate in seeds and help to improve the biochemical and germination parameters (Warwate *et al*., 2017). In bio-priming, seeds treated with endophytic bacteria act as growth regulators to monitor the growth of seeds (Nayaka *et al.,* 2008).

Endophytic bacteria are the specific type of bacteria which are used to solubilize the organic as well as inorganic phosphorus and other microorganisms (Hilda & Fraga, 2000; Khiari & Parent, 2005). Solubility of phosphorus is determined by capability of microbes to breakdown the organic and inorganic compounds and release metabolites and bring them in soluble form by different chemical reactions. Because of biopriming, emergence of seedling becomes rapid and useful under severe soil and environmental conditions (Rao *et al.,* 2009). The control of soil borne pathogens relies mostly on pesticides that produce risks to the human health and ecological contamination. Biological seed covering and biopriming seed handlings are acquisition status in colonization of many plant pathogens that act as alternatives of chemical fungicides in present times.

Therefore, current study was designed to evaluate the treatment of seeds with different endophytic bacterial strains to improve the seed germination, seedling parameters and biochemical changes induced by pre-

sowing seeds treated with endophytic bacteria. It is hypothesized that priming with bioagents would improve the germination as well as defense mechanisms against reactive oxygen species (ROS).

Materials and Methods

Taxonomically identified hybrid varieties (FH545, FH615 and FH620) of sunflower (*Helianthus annuus*) were taken from Oil Seed Center, Ayub Agriculture Research Institute, Faisalabad, Pakistan.

Seed priming with bioagents and germination parameters: Sunflower hybrid seeds (FH545, FH615 and FH620) were treated with selected endophytic bacterial species, *Enterobacter* sp. (FD-17), *Burkholderia phytofirmans* (PsJN) and *Bacillus* sp. (KS-54) that was previously isolated from different sources obtained from Soil Microbiology and Biochemistry Lab, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. About 10-15 mL of selected bacterial culture was taken and diluted at 1:10 ratio in petri plate. Seeds were dipped in the culture for 12 hours under optimized conditions (at room temperature). After treatment, seeds were grown (in petri plates) under germination incubator in Department of Crop Physiology, University of Agriculture, Faisalabad, Pakistan. All the experiments were designed in replicates and germination data was taken at 7th day of sowing the seeds using following parameters.

Germination percentage was calculated at 7th day of incubation by the process designed by Ijaz *et al*., (2012).

$$Gp = (Ng/Np) \times 100$$

where, Ng is the last number of emerged seeds and Np is the total number of seeds sown.

Mean growth time (MGT) in days was calculated as follows:

$$MGT = \sum(Dn)/\sum n$$

where n is number of seeds germinated on day D, and D is number of days counted from the beginning of the germination test.

Seedling vigor was calculated by the equation used by Vashisth & Nagarajan (2010).

Vigor index I = Germination (%) × Seedling length (root + shoot)
Vigor index II = Germination (%) × Seedling dry weight (root + shoot)

Extraction of antioxidant enzymes: The sunflower seedlings (0.1 g) were mixed with 50 mM phosphate buffer (pH 7.5) and centrifuged at 10,000 x*g* for 10 min to extract proteins and then filtered for enzyme activities (Zaheer *et al.,* 2012).

Biochemical studies of extracts: Following biochemical activities were performed.

Determination of enzyme assays: Catalase activity was measured using the method described by Sahu *et al.,* (2010) with little modification. For estimation of catalase (CAT), seedlings of sunflower were homogenized in phosphate buffer (50 mM) having pH 7.1 and mixed with reaction mixture containing H_2O_2. Absorbance was measured at 240 nm. Peroxidase assay was performed using method described by Chance & Maehly (1955) with minor modification. Reaction mixture contained H_2O_2 (30 mM) and guaiacol and 0.05 mL enzyme extract. Absorbance was taken at 470 nm. The standard procedure adopted by Manafa *et al*., (2017) was used for the calculation of superoxide dismutase (SOD) by measuring the photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm. The standard methods used by Bradford (1976) and Pandey and Budhathoki (2007) were adopted for the quantitative estimation of soluble proteins using bovine serum albumin (BSA). Absorbance was measured at 595 nm. Units of enzyme activity were compared with amount of total soluble proteins that was detected in sample extract.

Malondialdehyde lipid peroxidation (MDA): Plant seedlings (10 mg/mL) were ground in phosphate buffer with equal volume of (0.5% w/v) thiobarbituric acid in 20% (w/v) TCA. The mixture was cooled rapidly after the incubation for 30 min at 95°C in water bath. The filtrate was used for determination of MDA content by taking absorbance at 532 nm (Oracz & Bailly, 2007).

Protease activity: Standard methods described by Ahmed *et al*., (2011a and 2011b) and Iqbal *et al*., (2011) were used for the measurement of protease activity.

Amylase activity: Amylase activity was determined using the method of Kazeem *et al*., (2013). In this procedure 250 µL of enzyme extract was taken in 0.02 M sodium phosphate buffer (pH 6.9) containing α- amylase solution (0.5 mg/mL) and incubated for 10 min followed by adding 1% starch solution. The reaction was ceased using dinitrosalicylic acid (DNS). Absorbance was taken by ELISA reader at 540 nm.

Phenolic profiling through HPLC: Phenolic profiling was carried out using the methods adopted by Hussain *et al*., (2012). Phenolic acid contents present in the extract were measured using gradient scheme of HPLC using reverse phase column (C18). The mobile phase was 70% acetonitrile in methanol that was considered as solvent A and 5% glacial acetic acid considered as solvent B and the flow rate was set to 1 mL/min in gradient mode. The gradient scheme was as follows; solvent A (100%) for first 5 minutes, solvent A (95%) and solvent B (5%) for 5-10 minutes, solvent A (5%) and solvent B (95%) for 10-30 minutes, and solvent A (100%) from 30-35 minutes. The sample (20 µL) was loaded through HPLC sample port using micro syringe. Detection was carried out with built-in UV-visible spectroscopic detector at 275 nm.

Statistical analysis: The "Design expert" statistical software (version 7) was applied for the germination parameter analysis. Two-way analysis of variance (ANOVA) was carried out for all the biochemical parameters using Graph Pad Prism (version 5).

Results

Biopriming using bacterial strains as biocontrol agent pretreatment of sunflower seeds induced significant changes in physiological characteristics and the growth of seedlings. The growth parameters of seedlings (percentage of mean germination, mean germination time, vigor index I and II etc.) were measured (Fig. 1).

Effect of bioagents on seed growth: Data in Table 1 presented the germination parameters of sunflower seed varieties. All growth-related parameters were calculated as mean of triplicate experiments and standard deviation (mean ± SD). The significant difference in treatment using different bioagents (bacterial agents) was calculated at $p<0.05$. In FH620 variety it was found that seeds treated with bacterial agents gave 100% germination percentage (GP) and maximum percentage of mean germination time (MGT). Seeds treated with *Enterobacter* (FD-17) exhibited higher vigor index I and II as compared to unprimed seeds. In case of seed variety FH615 again *Enterobacter* (FD-17) gave maximum germination percentage and mean germination time (MGT) that was followed by *Bacillus* sp. (KS-54). Same result was found in case of FH545 variety but its mean germination percentage was decreased up to 30% as compared to other varieties. It was observed from germination parameters that seed variety FH620 gave high germination percentage when treated with *Enterobacter* (FD-17) that was comparable with other bacterial strains as well as un-primed seeds.

From the plots of response surface methodology (RSM) it was showed that seed variety of FH620 and FH615 exhibited higher mean germination time as compared to FH545. The later showed low mean germination time when treated with *Enterobacter* (FD-17). Hybrid variety FH620 of sunflower seeds showed optimum germination rate which was 40% after biopriming with *Enterobacter* (FD-17) as compared to 30% of untreated seeds.

Biochemical analysis of seed varieties of sunflower: Biochemical analysis was performed to study the effect of plant growth promoting regulators (biocontrol agents) on different hybrids of sunflower seedlings.

A significant increase in catalase activity was observed in seeds treated with bacterial strains as compared to control (unprimed) seeds. From the Fig. 2(a), it was shown that maximum catalase activity was noted in FH620 hybrid variety treated with *Enterobacter* (FD-17) at concentration of 500 µg/µL (showed catalase activity 0.844±0.34 IU/mg and control group showed catalase activity 0.772±0.07 IU/mg of protein) as compared to other bacterial (KS-54 and PsJN) agents. FH615 hybrid variety when treated with *Enterobacter* (FD-17) at concentration (500 µg/µL) showed catalase activity of 0.752±0.34 that was compared with control group having catalase activity (0.743±0.07) IU/mg of protein. In case of FH545 catalase activity (0.811±0.04) IU/mg of protein was measured. From two-way analysis of variance (ANOVA) having $p<0.05$ showed that there was no significant difference among the catalase values noted among the seed hybrid varieties. The comparative increasing order of catalase activities by treatment with *Enterobacter* (FD-17) was found to be FH620>FH545>FH615>control group.

In Fig. 2(a) sign '**' indicates significant difference among catalase activities of seed varieties against bioprimed agents. FH620 variety showed significant change in catalase activity against FD-17 (*Enterobacter*) as compared to KS-54 (*Bacillus* sp.) and (*B. phytofirmans*) PsJN. In case of control group the seed varieties FH615 and FH545 exhibited significant values of catalase activities as compared to primed groups. Fig. 2(b) is showing the peroxidase activity of seed varieties. From this graph it was found that seed variety FH545 had higher peroxidase value (0.121±0.7) against (*B. phytofirmans)* PsJN indicated by '**' sign as compared to other bioprimed agents. The FH620 variety had high peroxidase value (0.122±0.9) against (*Enterobacter*) FD-17 that was indicated in Fig. 2(b) by '**' sign. While untreated group of seed varieties had low value of peroxidase as compared to treated group.

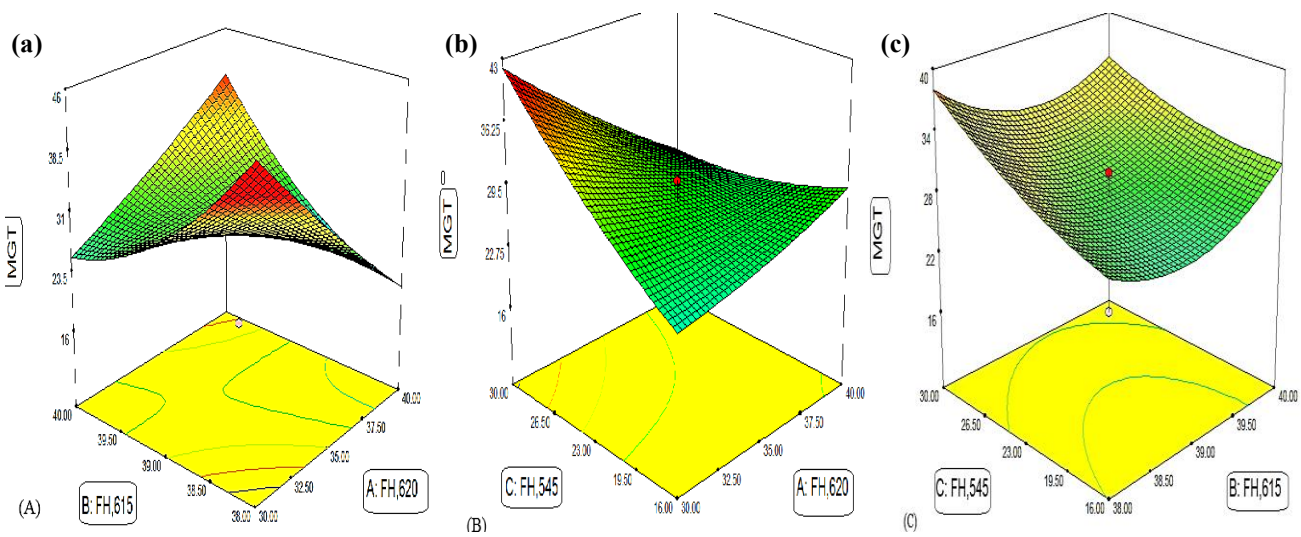

Fig. 1. Response surface plots showing the percentage of mean germination time of different seed varieties and their interactions (a) FH620, (b) FH615, (c) FH545.

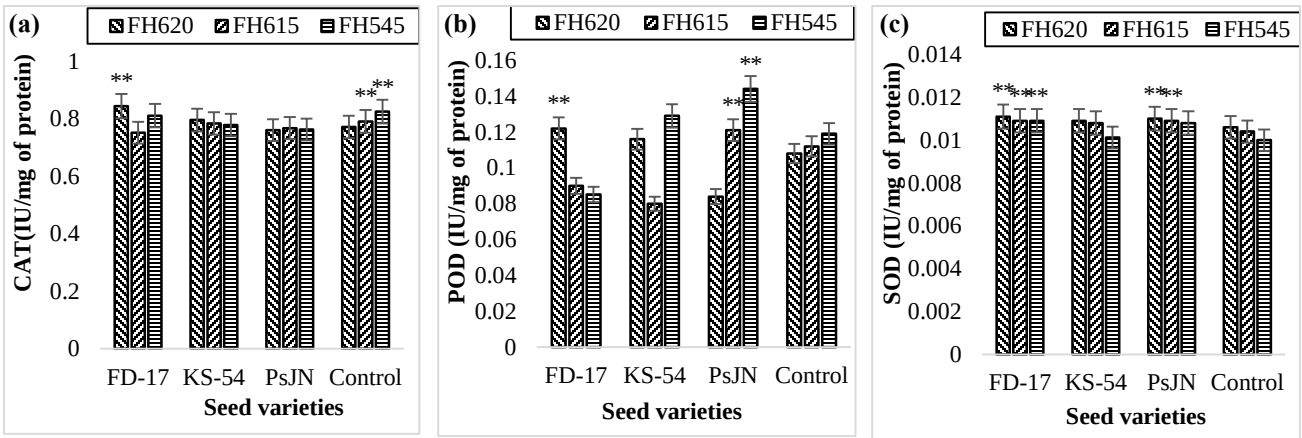

Fig 2. Biochemical study of sunflower seed varieties against bio-primed agents. (a) Catalase activity (IU/mg of protein), (b) peroxidase activity (IU/mg of protein), (c) superoxide dismutase activity of seed varieties against bioprimed agents.

Table 1. Effect of bioagents on germination parameters of sunflower seeds.

Seed varieties	Treatments	%age of germination	% age of MGT	Vigor index I	Vigor index II
FH620	*Enterobacter* (FD-17)	100 ± 1.00^{A}	40 ± 0.06^{A}	80 ± 0.16^{C}	7.00 ± 0.24^{C}
	Bacillus sp. (KS-54)	100 ± 1.00^{A}	40 ± 0.46^{A}	70 ± 0.42^{D}	4.00 ± 1.04^{E}
	B. phytofirmans (PsJN	100 ± 1.00^{A}	40 ± 0.59^{A}	27 ± 0.91^{F}	4.00 ± 1.42^{E}
	Control	75 ± 0.89^{C}	30 ± 0.82^{C}	80 ± 0.61^{C}	8.25 ± 1.00^{B}
FH615	*Enterobacter* (FD-17)	100 ± 1.00^{A}	40 ± 0.24^{A}	120 ± 0.80^{A}	8.00 ± 1.24^{B}
	Bacillus sp. (KS-54)	85 ± 1.4^{B}	38 ± 0.83^{B}	40 ± 0.20^{E}	6.65 ± 1.00^{D}
	B. phytofirmans (PsJN)	80 ± 1.7^{C}	40 ± 0.40^{A}	85 ± 1.49^{B}	6.40 ± 0.67^{D}
	Control	75 ± 0.89C	30 ± 0.24^{C}	35.25 ± 1.36^{E}	15.75 ± 0.24^{A}
FH545	*Enterobacter* (FD-17)	80 ± 1.67^{C}	32 ± 0.51^{C}	88 ± 0.37^{C}	6.4 ± 0102^{D}
	Bacillus sp. (KS-54)	75 ± 0.84^{C}	30 ± 0.83^{C}	82.5 ± 1.15^{C}	15.75 ± 1.00^{A}
	B. phytofirmans (PsJN)	75 ± 0.86^{C}	30 ± 0.44^{C}	112.5 ± 1.49^{B}	7.40 ± 1.24^{C}
	Control	40 ± 0.67^{F}	16 ± 0.87^{F}	26 ± 0.08^{F}	7.20 ± 0.32^{C}

The data presented in the table are the mean values of three replications ± stander error

Table 2. Phenolic acid profile of seed hybrid varieties (FH545, FH615 and FH620) of sunflower.

Sr. No.	Phenolic compounds	Retention time	Indication of phenolic compounds in sunflower hybrid varieties			
			Control	FH620	FH615	FH545
1.	Caffeic acid	12.64	✓	✓	✓	X
2.	Benzoic acid	12.92	✓	✓	✓	X
3.	Chlorogenic acid	15.75	X	✓	X	✓
4.	Syringil acid	20.35	X	✓	✓	✓
5.	M-coumaric acid	22.54	✓	✓	✓	✓
6.	Ferulic acid	24.94	X	✓	X	X

Fig. 2(c) represents superoxide dismutase activity of seed varieties of sunflower against bioprimed agents. It was found that FH620 variety of seed showed highest value of superoxide dismutase against all bioprimed agents including (*Enterobacter*) FD-17, (*Bacillus* sp.) KS-54 and (*B. phytofirmans*) PsJN having (0.011±0.009 IU/mg) of protein at 500 µg/µL concentration. While FH615 and FH545 indicated high value of superoxide dismutase against (*B. phytofirmans*) PsJN having (0.0109±0.05 IU/mg) of protein of superoxide dismutase. It was noted that superoxide dismutase values were higher as compared to unprimed (control) group. Significant difference was presented by '**' sign. All the results were calculated by two-way analysis of variance (ANOVA) having $p>0.05$.

It was indicated from Fig. 3(a) that treatment of seeds of FH615 with bioprimed agent (*Enterobacter*) FD-17 had protein contents (22.43±0.48 mg/mL) that was followed by FH620 variety having (17.69±1.02 mg/mL) protein contents at higher concentration (500 µg/µL). From this, it was indicated that *Enterobacter* (FD-17) treatment gave higher protein contents as compared to other bioprimed agents while FH545 gave (18.72±0.89 mg/mL) of protein against KS-54 (*Bacillus* sp.). It was noted that all seed varieties had higher total soluble protein contents as compared to controlled group. The significant difference was indicated by '**' signs.

Malondialdehyde lipid peroxidation parameter was calculated against bioprimed agents of sunflower seed varieties (Fig. 3b). FH620 seed variety when treated with

FD-17 (*Enterobacter*) showed higher MDA value (95.52±0.75) followed by FH615 when treated with KS-54 (*Bacillus* sp.) having (82.35±0.78 mg/mL) of MDA value. In case of FH545 when treated with FD-17 (*Enterobacter*) had (67.85±0.75 mg/mL) MDA at 500 µg/µL concentration while control group of all these seed varieties have low MDA value.

Percentage inhibition of amylase activity of sunflower seed varieties was also determined (Fig. 4). It was found that FH615 seed variety when treated with FD-17 (*Enterobacter*) showed higher (85.97±1.23) percentage inhibition of amylase activity at 500 µg/µL concentration followed by FH620 when treated with PsJN (*B. phytofirmans*) having (84.45±1.43) percent inhibition of amylase activity (Fig. 4a). In case of control group FH545 showed higher percentage of amylase activity as compared to treated group while FH620 and FH615 control groups showed low percentage of inhibition of amylase activity. Significant difference was indicated by '**' sign compared by two away analysis of variance (ANOVA).

Significance difference was indicated by '**' sign. Fig. 4(b) represents the percentage of protease activity. It was found that FH620 variety of seed when treated with KS-54 (*Bacillus* sp.) exhibited (10.33±0.40) protease activity followed by FH545 treated with FD-17 (*Enterobacter*). In case of unprimed (controlled group) it was found that seed variety of FH615 had (9.66±1.23) percentage of protease at 500 µg/µL concentration. Significant difference was presented by '**' sign.

HPLC analysis for phenolic profiling: The qualitative and quantitative HPLC analysis of control and primed-seed hybrid varieties (FH545, FH615 and FH620) extracts was carried out using gradient elution system. Prior to the sample elution, the solution of standard phenolic acids (i.e. Gallic acid, cinnamic acid, ferulic acid, benzoic acid, quercetin, sinapic acid, vanillic acid, chlorogenic and *p*-coumaric acid) were eluted under same conditions of temperature, pressure, flow rate and solvent system. The qualitative information about the phenolic acid profiling of extracts of control and selected seed varieties are shown in HPLC chromatograms (Fig. 5). The presence of phenolic compounds detected in hybrid seed varieties of sunflower are mentioned in Table 2.

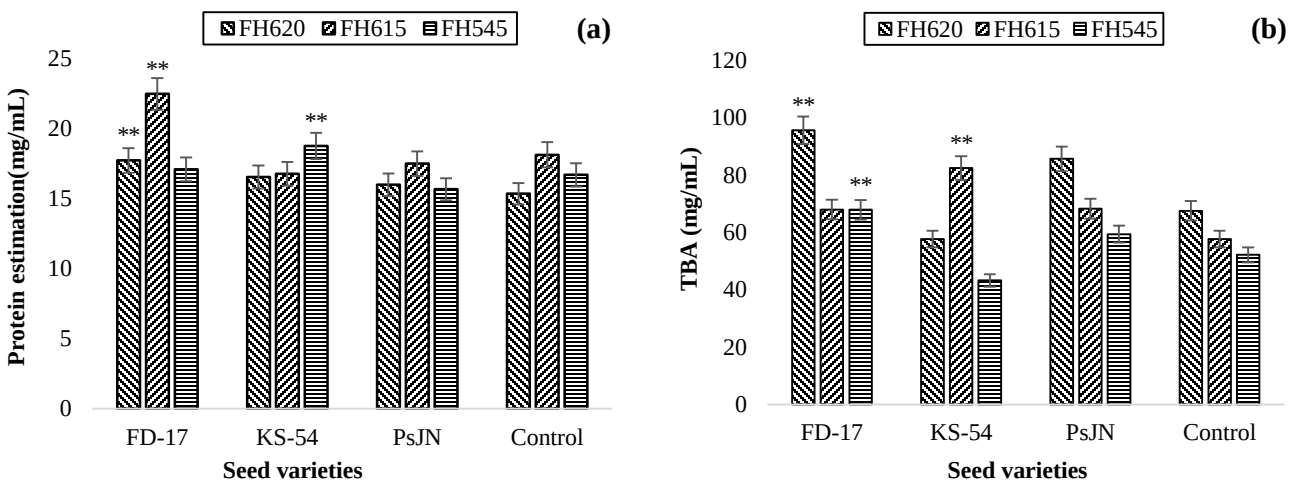

Fig. 3. Estimation of total soluble protein and Malondialdehyde lipid peroxidation (MDA) of seed varieties of sunflower. (a) total soluble protein (mg/mL), (b) malondialdehyde peroxidation (mg/mL of TBA) of seed varieties against bio-primed agents. Significant difference is indicated by '**' sign

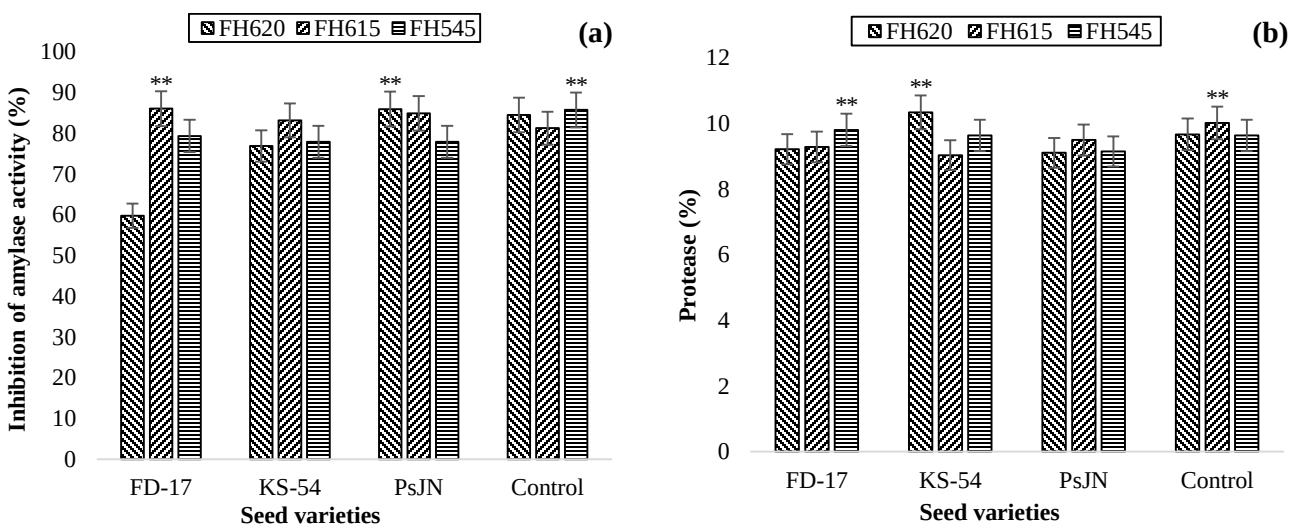

Fig. 4. Percentage inhibition of amylase activity and protease activity of seed varieties against bio-primed agents. (a) percentage inhibition of amylase, (b) percentage of protease of seed varieties against bioprimed agents

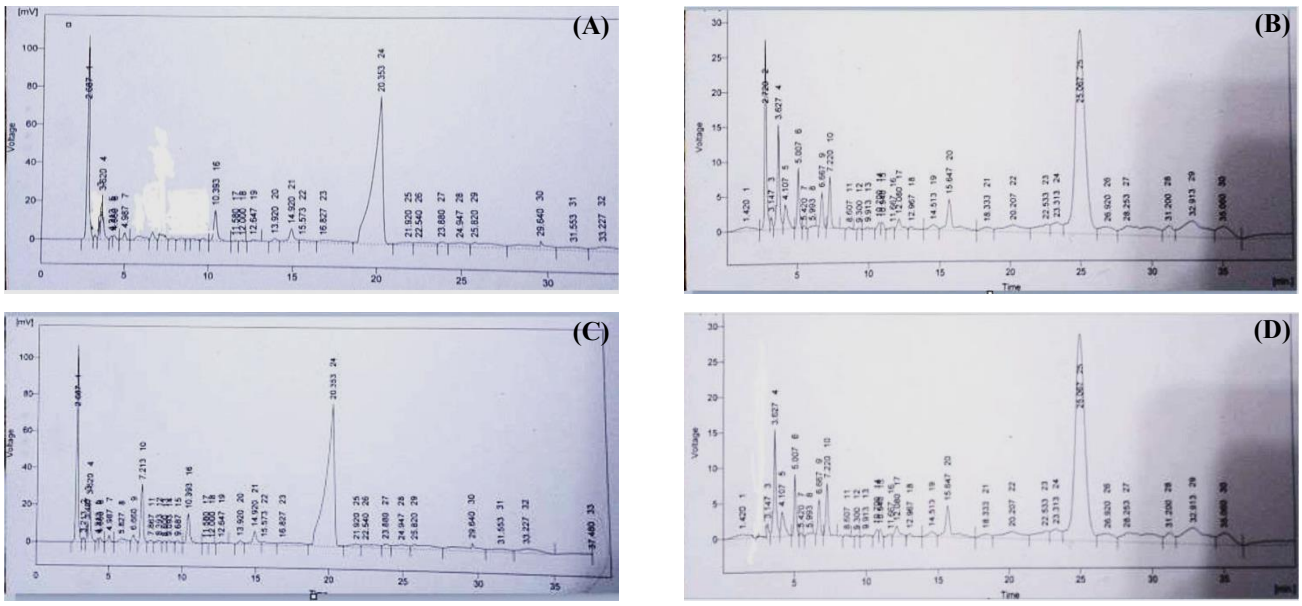

Fig. 5. HPLC analysis of controlled and primed-seed sample. (A) FH620, (B) FH615, (C) FH545, (D) Controlled group analysis of sunflower seeds for phenolic profile

Discussion

This study was aimed to analyze growth, biochemical and phenolic composition of sunflower seed hybrid varieties (FH545, FH615 and FH620) an important oil seed crop by priming its seeds with different endophytic bacterial agents prior to sowing. Treatment with endophytic bacteria plays a significant role on sunflower physiology as well as its biochemistry. These endophytic bacteria play an important role in growth of the plants and therefore called as plant growth promoting regulators (PGPR). These bacteria affect plant growth advancement process such as increased solubilization and uptake of nutrients and/or production of plant growth regulators. It was found that high range of final emergence percentage is relate with higher water potential along with endophytic bacteria for short period of time but it gives negative impact when treated with low water potential for long period of time (Ihsan *et al.,* 2011).

Effect of endophytic bacterial strains on growth was studied using different bacterial strains including *Enterobacter* sp. (FD-17), *B. phytofirmans* (PsJN) and *Bacillus* sp. (KS-54). The result indicated that FH620 variety of sunflower seeds primed with *Enterobacter* sp. (FD17), *B. phytofirmans* (PsJN) and *Bacillus* sp. (KS-54) treatment gave significantly (p<0.05) high germination percentage (100±1.00) and mean growth time (MGT) (40±0.06) as compared to unprimed as well as other FH615 and FH545 seed varieties. Similarly, seedling vigor (i.e strength of plant growth of FH615) was more effected by FD17 (*Enterobacter*) (p<0.05) followed by FH545 when treated with (*B. phytofirmans*) PsJN endophytic bacteria. Different species of endophytic bacteria have been reported to play important role in effective growth elevation of sunflower seedlings (Raj *et al*., 2004). Moreover, it was found that growth promoting bacteria can improve the growth and biomass production in different plant species including sunflower (Mafia *et al*., 2009). Rhizobacteria which are found in rhizosphere of plants are an important and active component of microbial population (Aslam *et al*., 2018). In this respect, one study pointed out that some species of *Pseudomonas* spp. promote plant growth by increasing nutrient absorption of nitrogen, potassium and phosphorus and provide hormones in the rhizosphere which defend against phytopathogenic organisms (Díaz *et al.,* 2001; Duda, 2004).

Biopriming with selected endophytic bacterial strains (FD17, KS54 and PsJN) meaningfully improved the growth attributes including root length, shoot height, dry and wet weight, vigor index I and II of seedlings over hydropriming. This might be because bacteria attached with seeds before planting colonize properly and affect the traits. The bioprimed seeds grew rapidly as compared to control. The main advantage of biopriming is that bacterial population gets reduced during bioprimed seeds. The similar results were presented by Callan *et al*., (1990) who reported that seed hydrated after treatment with endophytic bacteria emerged at a higher rate than seed hydrated after only surface-disinfestation.

Reactive oxygen species (ROS) are chemically volatile molecules that have ability to form free radicals which result in damaging of cells. When one electron is added to two oxygen atoms then as a superoxide anion radical (O_2^-) is formed. Hydroxyl radical, singlet oxygen, hydrogen peroxide, superoxide anion radical and oxides of nitrogen and hypochlorite are different types of ROS. The cellular damage takes place as a result of ROS reaction with membrane, lipids, proteins and other biological parts (Manafa *et al.,* 2017). Natural antioxidants are gaining importance because of their health improving qualities and have been used in foods, as therapeutic agents and preventive medicines (Atta *et al*., 2017; Sharif *et al*., 2018). Biochemical analysis was done to study the effect of plant growth promoting regulators (biocontrol agents) on different hybrids of sunflower seedlings. This enzyme is important for removal and detoxification of hydrogen peroxide at seedling stage. An increased level of enzyme protects the cell from oxidative damage (Zhang *et al.,*

2007). From Fig. 2(a) it was found that there was little significant difference in catalase activity among all selected varieties of seeds when treated with endophytic bacteria except unprimed seeds. This is due to the fact because these endophytic bacteria increase the solubilization of nitrogen, phosphorus and potassium that play its role as a catalyst or part of catalase enzyme which result to increase the catalase activity of treated seedlings. But there was a minor change observed in the treated and uncontrolled samples (Luhova & Hedereerova, 2003). Catalase is essential enzyme for removal/ detoxification of hydrogen peroxide in seeds. An increase in the level of this enzyme protects cells from oxidative damage (Zhang *et al.,* 2007).

From Fig. 2(b) peroxidase activity of sunflower hybrid seed varieties was measured and FH545 treated with (*B. phytofirmans*) PsJN showed significant value of peroxidase. The fact is that peroxidase activity begins to develop in germinating seeds without any environmental facts. Alternatively, this peroxidase activity protects the germinating seeds from pathogens. In case of sunflower, maize and many other crops, the presence of H_2O_2 helps to protect from pathogens at early stages.

FH615 seed hybrid variety gave highly significant result when treated with FD17 (*Enterobacter*) as compared to others seed varieties. This was because of solubilization of minerals that increase formation of protein as storage amount of lipids and proteins increase (Sarmadi & Ismail, 2010). In present work, association between the loss of seed capability and the gathering of MDA was not observed. The gathering of MDA is related with lipid peroxidation because of the attack of reactive oxygen species on polyunsaturated fatty acids, leading to seed weakening and reduced capability of growth (El-Maarouf *et al.,* 2011). The gathering of MDA during ageing is stated in several species including sunflower (Kibinza *et al.,* 2006), wheat (Chen *et al.,* 2018), cotton (Goel *et al.,* 2003), and soybean (Sharma *et al.,* 2013) during artificial and natural ageing. Despite these explanations Kibinza *et al*., (2006) also observed at low moisture contents that level of malondialdehyde decreases in seeds of sunflower when stored. Activation/ production of enzymes occurred as a result of seed enhancement which is necessary for the mobilization of seed reserves (Subramani *et al.,* 2011). Between these actions, breakdown of carbohydrate (starch) contents is controlled by α-amylase and breakdown of protein by proteases (Bishnoi *et al.,* 1993) and similarly for hydrolysis of ester group of enzymes which is called as esterase (Subramani *et al.,* 2011). Increased level of α-amylase activity in the seeds is responsible for increase in level of non-reducing sugars in seeds by biopriming. Amylase is the most important enzyme that helps to breakdown the starch contents of seeds. Current results have shown that seed priming grouping has elevated the amylase activity in seeds. Farooq *et al.* (2008) have discussed the amylase activity using different priming techniques.

Basra *et al*., (2005) reported a significant increase in the amylase activity of treated seeds with bio-priming technique as compared to control (untreated). An increased in phenolic acid concentration in primed seeds lead to increase the biochemical profiling of sunflower hybrid seed varieties.

Conclusion

It was concluded that among endophytic bacteria, *Enterobacter* (FD17) showed more effective results on the sunflower seeds, germination parameters as well as biochemical profiling. Pre-sowing sunflower seeds treated with microbes positively affect the plant (seed) germination and development stages which lead to accelerate germination, yield and an increased biochemical profiling which in turn increases the efficacy of seeds. From this study it was found that biochemical profiling including catalase, peroxidase, superoxide dismutase, amylase and protease showed more significant results of primed seed varieties as compared to un-primed ones. The data approach of this study was further used not only to improve the germination parameters but also to increase the yield level and prevent the seeds from decaying.

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