*J. Plantarum.*, 6(1): 102-129

ISSN PRINT 2710-4079
ISSN ONLINE 2710-4087

# ASSIMILATED EVALUATION OF PLANT GROWTH-PROMOTING RHIZOBACTERIA ON SEED-BORNE MYCOFLORA AND SEED VIGOUR ASSOCIATED WITH COMMERCIAL VARIETIES OF WHEAT (*TRITICUMAESTIVUM* L.)

SHAMA SHARIF[1], ZILL-E-HUMA AFTAB[1*], TEHMINA ANJUM[1], MAROOF SIDDIQUE[1], WAHEEDAKRAM[1]

*Department of Plant Pathology. Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.*
*1Department of Plant Pathology. Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.*
**Corresponding author email: huma.dpp@pu.edu.pk*

Received on: 10-12-2023 Reviewed on: 03-06-2024 Accepted on: 06-06-2024 Published on: 20-06-2024

## Abstract

Among all cereal crops, wheat is the most important staple food globally and in Pakistan. In this study, two parameters, mainly the effect of PGPR inoculation on seed germination, emergence and overall seed vigour under different temperature conditions and wheat associated seed-borne mycoflora in 10 commercial wheat cultivars, were investigated. A temperature of 20 °C was found to be more effective than 15 °C and 25 °C, which showed maximum seed germination and seed vigour. Ojala 2016 and Johar 2016 were found to have 100% germination percentage at 20 °C in seeds inoculated with a dual bacterial inoculum. Ojala 2016 in the dual inoculation (PGPR 2) experiment had maximum shoot and root length (5.67 cm and 8.07 cm). Seed-borne mycoflora associated with 10 varieties of wheat were examined through the blotter paper method and agar plate method. Eleven fungal species were isolated and identified from seeds. Fungal species were identified using microscopic identification techniques. Agar plate method was found to be most efficient for fungal isolation. Plant fight against soil-borne pathogen invasions through root exudates by recruiting beneficial microorganisms, that is plant growth-promoting rhizobacteria (PGPR), which provide bioactive and antibacterial compounds to manage various phytopathogens. Fungal incidence was higher in unsterilized seeds than in sterilized seeds. The most frequently occurring fungal species were *Penicillium* (46%, 43%) and *Aspergillus niger* (40%, 56%) in sterilized and unsterilized treatments of blotter paper. In agar plate method, *Penicillium* (76%, 76%) and *A. niger* (70%, 83%) were frequently occurring species. Sahar 2008 and Aas 2011 showed maximum contamination among all ten varieties of wheat.

**Key words:** Blotter paper, dual inoculation method, seed vigour, fungal incidence, agar plate method and PGPR inoculation.

## Introduction

Wheat (*Triticum aestivum* L.), known as bread wheat, is among the most important cereal cropsworldwide and serves as a staple food for 30 percentof the human population (International Wheat

Genome Sequencing et al., 2014). Wheat is Pakistan'sprincipal agricultural crop. On an area of roughly ninemillion hectares, or close to 40% of the nation's total arable land, it is anticipated that 80% of Pakistani farmers grow wheat (Rasheed et al., 2021). Wheat hasa significant impact on Pakistan's GDP and increases the country's revenue from foreign exchange. According to figures from the Government of Pakistan (GOP), wheat specifically contributes 8.9% of thevalue added in agriculture and 1.6% of GDP (Rehmanet al., 2015).

Numerous seed-borne pathogens have an impact on the wheat crop, which has the potential to significantly reduce global production. Wheat in Pakistan is reported to be infected by 50 seed-borne phytopathogens. (Khan et al., 2023). *Aspergillus*, *Penicillium*, *Alternaria alternata*, *Curvularia lunata*, *Bipolaris*, *Fusarium graminearum*, *Drechsleria* and *Helminthosporium* are among the phytopathogens that usually account for wheat seed mycoflora

(Hussain etal., 2013; Mehboob et al., 2015; Pitt and Hocking, 2009).

Plant germplasm resources are viewed as the cornerstone of crop production and breeding. Additionally, they are vital strategic resources for human development and survival (Zafar et al., 2014). Using disease-free and certified seeds is the first step in managing plant diseases. The germination test provides important information on mycoflora and theirefficient treatment. It is also a significant step in identifying the seed-borne pathogen associated with wheat seeds (Anna, 2016; Pathak and Zaidi, 2013).

For poor stand establishments, priming is a low-risk and low-cost technique. The use of seed priming techniques enhances the crop's germination, seedling emergence, growth, and yield characteristics(Hussian et al., 2013). The pharmaceutical business inspired the first artificial seed coating on cereal seedsin the 1930s, and in the 1960s, large-scale commercialuse of this technique began (Kaufman, 1991). Plant protection, lowering environmental stress, or enhancing plant development are some of the functions of seed coating based on its mechanism of action or physical characteristics (Amirkhani et al., 2016). Furthermore, priming tomato cultivars with polyamines has been said to improve germination andseedling vigour (Hameed et al., 2013). There are several methods for priming seeds, which may be roughly categorized into advanced approaches (nano-priming and priming with physical agents) and classic methods (hydro-priming and osmo-priming, chemical priming, bio-priming, nutritional priming, and primingwith plant growth regulators). However, a number of variables, including seed characteristics, temperature, aeration, light, and priming duration, have a significant impact on priming (Waqas et al., 2019). In fact, seed coating is employed as a biological strategy to enhance agricultural sustainability (Paravar et al., 2023).

The coating process or seed treatment known as "bio-priming" optimizes the pre-germination procedures without radicle emergence by treating seeds with advantageous Plant Growth Promoting Rhizobacteria (PGPR) under controlled hydration circumstances (Sukanya et al., 2018). One of the less expensive and more environmentally friendly ways to boost a plant's early or primary growth stages is by bio-priming the seeds with PGPR (Deshmukh et al., 2020). *Pseudomonas* spp. (Chitra and Jijeesh, 2021), *Bacillus* spp. (Bidabadi and Mehralian, 2020), *Enterobacter* spp. (Roslan et al., 2020), and *Azospirillum* spp. (Gowthamy et al., 2017) are amongthe helpful PGPRs that can be used as a bio-inoculant or seed bio-priming agent to promote seedgermination, nutrient absorption, and stress tolerance. They are best suited for the biopriming method and give plants resistance to biotic stress because they produce plant growth regulators like auxins, abscisic acid, cytokinins, and gibberellins, as well as secrete secondary metabolites through modulation of various pathways and cascades (Mitra et al., 2021).

The aim of this research was to identify the mycoflora associated with the ten distinct wheat varieties that are currently on the market using methods from the International Seed Testing Association (ISTA). The findings would contribute to the collection of empirical data on the most prevalent genotypes and provide potential countermeasures for each genotype's associated diseases. Another objective was to analyze the response of wheat seeds to PGPR inoculation, one of the significant countermeasures to the imminent

threat of seed-borne fungi, and the performance of wheat under such a threat.

**Materials and Methods Collection of wheat seeds**

Commercial seeds of ten wheat varieties (i.e., 'Aas 2011', 'Millat 2011', 'Faisalabad 2008', 'Johar 2016', 'Galaxy 2013', 'Sahar 2008', 'Punjab 2011', 'Aari 2011', 'Ojala 2016', and 'Fakhar-e-Bhakkar 2016') were collected from private sector of Lahore, Pakistan. For seed vigor test, PGPR samples were brought to the Seed pathology laboratory at Faculty of Agricultural Sciences (FAS), University of the Punjab, Lahore.

**Procurement of PGPRs**

PGPR samples were procured from the Ayub Agriculture Research Institute (AARI), Faisalabad. In PGPR 1 (N2) treatment, *Azospirillum lipoferum* was used while PGPR 2 (N2+P) treatment consisted of dual inoculations of N-fixing bacteria (*Rhizobium leguminismarum*) and phosphorus-solubilizing bacteria (*Pseudomonas fluorescens*).

**Analysis of Seed-Borne Mycoflora**

In accordance with the International Rules of Seed Testing (ISTA, 2023), two separate detection standard methods; blotter paper (Habib et al., 2011; Mancini et al., 2016) and agar plate method (Demeke et al., 2005; Khan et al., 2023) were used to identify the mycoflora associated with seed samples. Each method, consisted of two types of seeds (i.e., sterilized seeds and unsterilized seeds). Three replicates from each sample were examined.

**Blotter paper method**

Aseptically, grains were placed on moistened three layers of blotter paper in 9 cm sterilized Petri dishes after being surface sterilized for 2 minutes with1% sodium hypochlorite solution and then rinsed with distilled sterilized water. The remaining seeds used forunsterilized treatment were left untreated and placed on blotter paper. Each Petri plate contained 10 seeds, which were then incubated for 7–10 days at 25 °C under a 12-hour cycle of fluorescent light and darkness. When necessary, distilled water that had been sterilized was used to wet the blotter paper.Individual grains were analyzed using a stereo- binocular microscope. The fungi that were developingon the grains were either identified directly from the plates or sub cultured onto other suitable identification medium that is 2% Potato Dextrose Agar (PDA) (Abdullah and Atroshi, 2016).

**Agar plate method**

The PDA (Potato Dextrose Agar) plating approach (Ahmad et al., 2016) was used to get more consistent and better growth for different wheat mycoflora. 20 g of potato extract, 20 g of agar, and 20 g of dextrose were combined to produce the modified PDA. The whole medium was autoclaved before it was poured into Petri plates. Seeds were surface sterilized for 2 minutes with 1% sodium hypochlorite solution and then rinsed with distilled sterilized water. Using sterile forceps, sterilized seeds were placed on plates. The remaining seeds used for unsterilized treatment left untreated were also placed, 10 grains per Petri plate. All Petri plates were maintained at 25 °C for 6 days with 12 h of alternate fluorescent light and darkness cycles. To see the mycoflora inside the seed,the stereoscopic microscope was used to inspect each plate. Colonies arising from the grains on each medium were separated to freshly prepared, suitable media for identification.

**Fungal Identification and seed-borne mycoflora analysis**

Under a microscope, fungal structures were

identified and measured, and their colony morphology, spore features, and spore formation were compared to previously published literature, referencebooks and identification keys (Guo-yin et al., 2013; McClenny, 2007; Nyongesa et al., 2015; Pavlic et al.,2008; Pitt and Hocking, 2009).

Additionally, the mycoflora present in the seed batches was tested for its proportion of radial development. Colony forming unit (CFU)/g can be used to quantify the fungal load by agar plate method (Dilution plating). Dilution plating relies on serial dilution of sample. Percentage contribution, relative frequency (RF), and relative density (RD) were calculated by the formulae as described by Gaddeya etal. (2012), Adhikari et al. (2018) and Khaledi et al. (2021): Total number of CFUs of species Contribution (%)

**Evaluation of Seed Quality Parameters**

Two experiments were performed to assess seed quality parameters: the germination test and the seed vigour test. Seed quality parameter experiments were conducted at three temperatures: 15 °C, 20 °C, and 25 °C. In each temperature condition, three treatments (control, PGPR 1, and PGPR 2) were carried out. And each experiment was conducted in a completely randomized manner, with three replicates for each wheat variety. Wheat seeds were surface sterilized with 1% NaOCl for 1 minute and washed with distilled water 3–4 times. After that, seeds were soaked in two Plant Growth Promoting Rhizobia Solutions, PGPR 1 (N2) and PGPR 2 (N2+P), for 20 minutes. Seeds soaked in only distilled water were treated as control. Seeds used in each of the inoculated (PGPR 1 and PGPR 2) treatments and control were placed in sterilized Petri plates containing blotter paper. Proper moisture was provided, and it was incubated at room temperature for three days. Data on shoot length (cm), root length (cm), percentage of seed germination, and seedling length vigour index (SLVI) were determined after three days of incubation. Then,the percentage of seed germination and SLVI were calculated according to Alemu (2014) and Khaledi et al. (2021) as follows:

**SLVI=(mean of shoot length + mean of root length) × percentage of seed germination**

**Statistical Analysis**

R-studio software was mainly used for statistical data analysis. The recorded data on relative frequency was statistically analyzed through the Agricoale package using Tukey's HSD. Pearson's coefficient of correlation was calculated using the GGAlly package in R Studio (Uzair et al., 2022). SLVI values were analyzed mainly by two-way ANOVA, and values were plotted in a bar graph using Tukey's HSD. GG plot was used to express fungal distributionby violin plot graph.

**Results**

**Fungal Incidence and Overall Percentage Contribution**

On the basis of wheat varieties, different fungal incidence patterns were observed. More fungal incidence was observed on the agar plate method than on the blotter paper method, and more fungal infection was observed in unsterilized seeds than in sterilized seeds. The results showed that among all ten varieties of wheat, Sahar 2008, Aas 2011 and Aari 2011 showed the maximum fungal incidence in both methods. Minimum fungal incidence was observed in Fakhar-e- Bhakkar 2016, Punjab 2011 and Faisalabad 2008 in both methods. A total of 11 fungi were isolated from 10 varieties of wheat, including *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus* sp.,

*Alternaria alternata*, *Curvularia* sp., *Fusarium oxysporum*, *Fusarium* sp., *Penicillium*, *Macrophomina phaseolina* and *Mucor* sp. In the blotter paper method, Sahar 2008, Aas 2011 and Aari 2011 had fungal incidences of 63%, 53%, and 43% on sterilized seeds and on unsterilized seeds, the incidence patterns observed were 70%, 56%, and 46%,respectively.

In the agar plate method of sterilized seed treatment, Sahar 2008, Aas 2011 and Aari 2011 gave fungal infections of 86%, 73%, and 70%, respectively, and on unsterilized seed it was 100%, 90%, and 83%. In the blotter paper method, sterilized treatment constituted 28 fungal colonies, and unsterilized treatment constituted 36 colonies. And in the blotter paper method, *Aspergillus niger* had the highest percentage contribution value of 16% and 22% in sterilized and unsterilized treatments, respectively. And *Aspergillus fumigatus* and *Fusarium oxysporum* both had a lower percentage contribution in sterilized treatment, at 1.16%, and in unsterilized *Macrophomina phaseolina*, have lower contribution of 1.83% (Figure 1 and Figure 2).

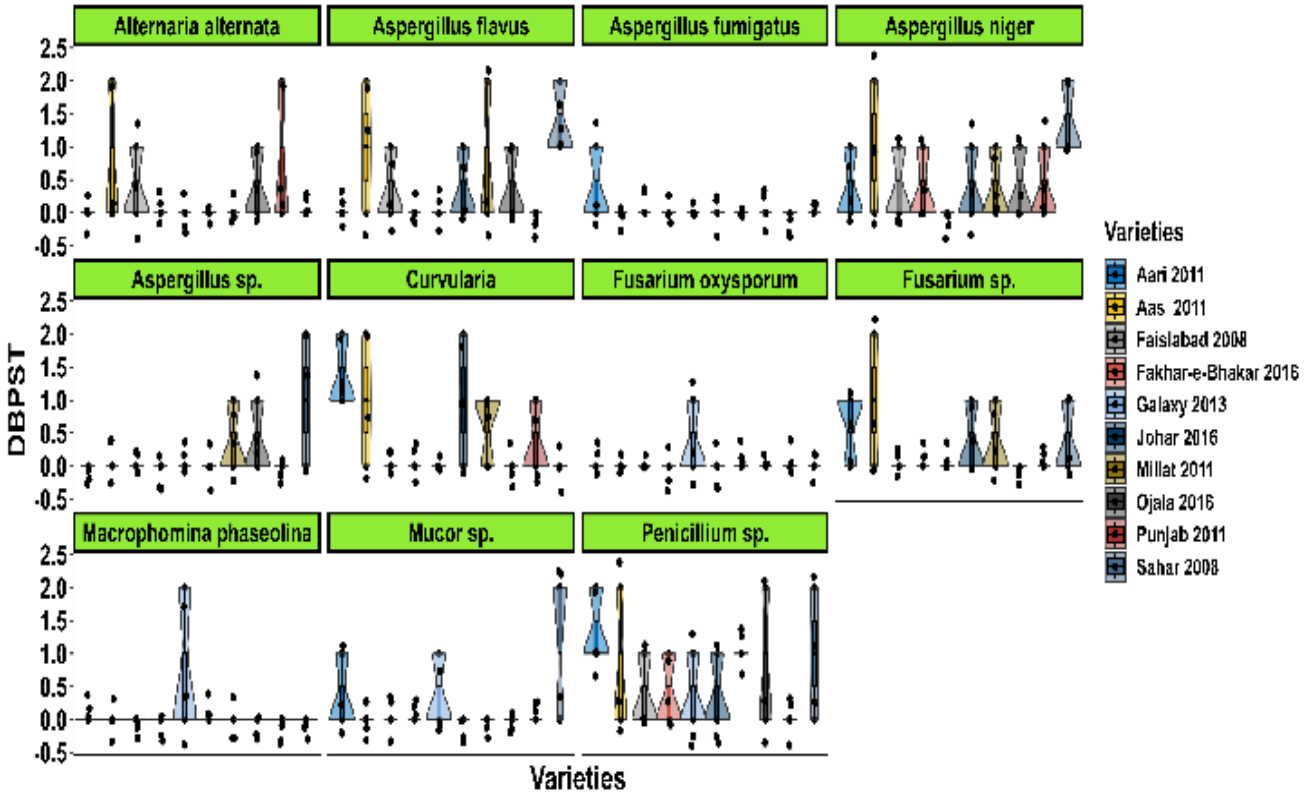


**Figure 1** Distribution of fungal mycoflora fromsterilized blotter paper method containing different wheat genotypes. Y-axis representing DBPST = distribution blotter paper sterilized method. Except Faisalabad 2008 and Fakhar-e-Bhakkar 2016 all genotypes showing maximum fungal distribution

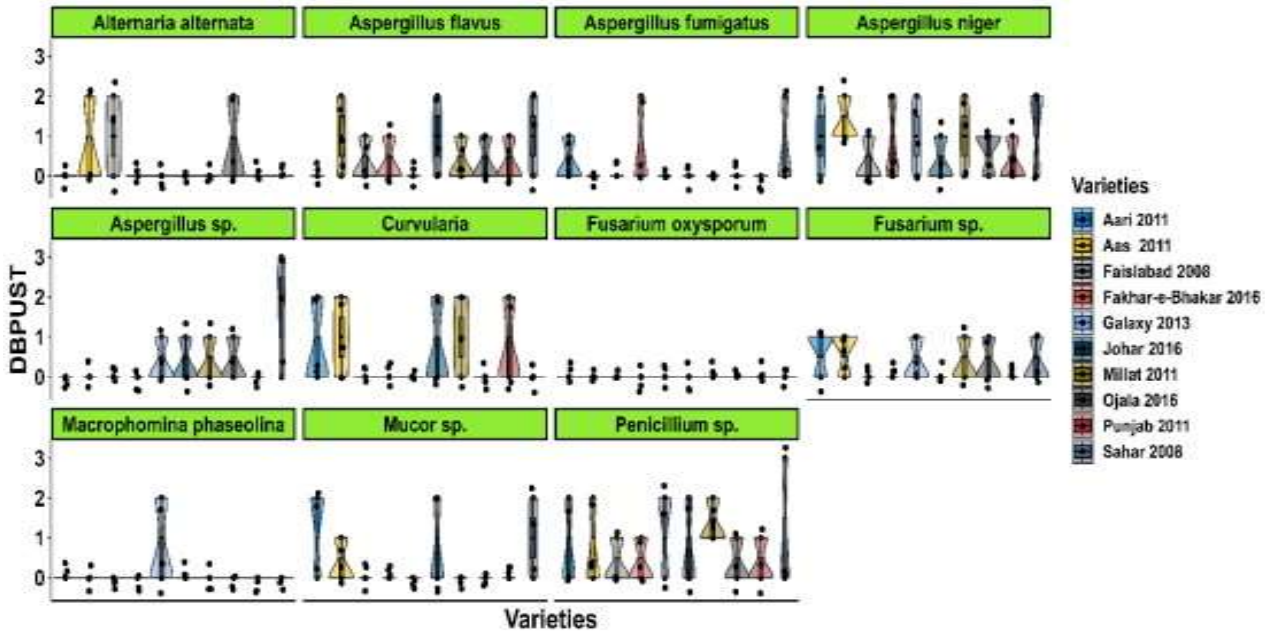


**Figure 2** Distribution of fungal mycoflora from unsterilized blotter paper method on different wheat genotypes. Y-axis representing DBPUsT = distribution blotter paper Unsterilized method. Sahar 2008 showing maximum fungal distribution

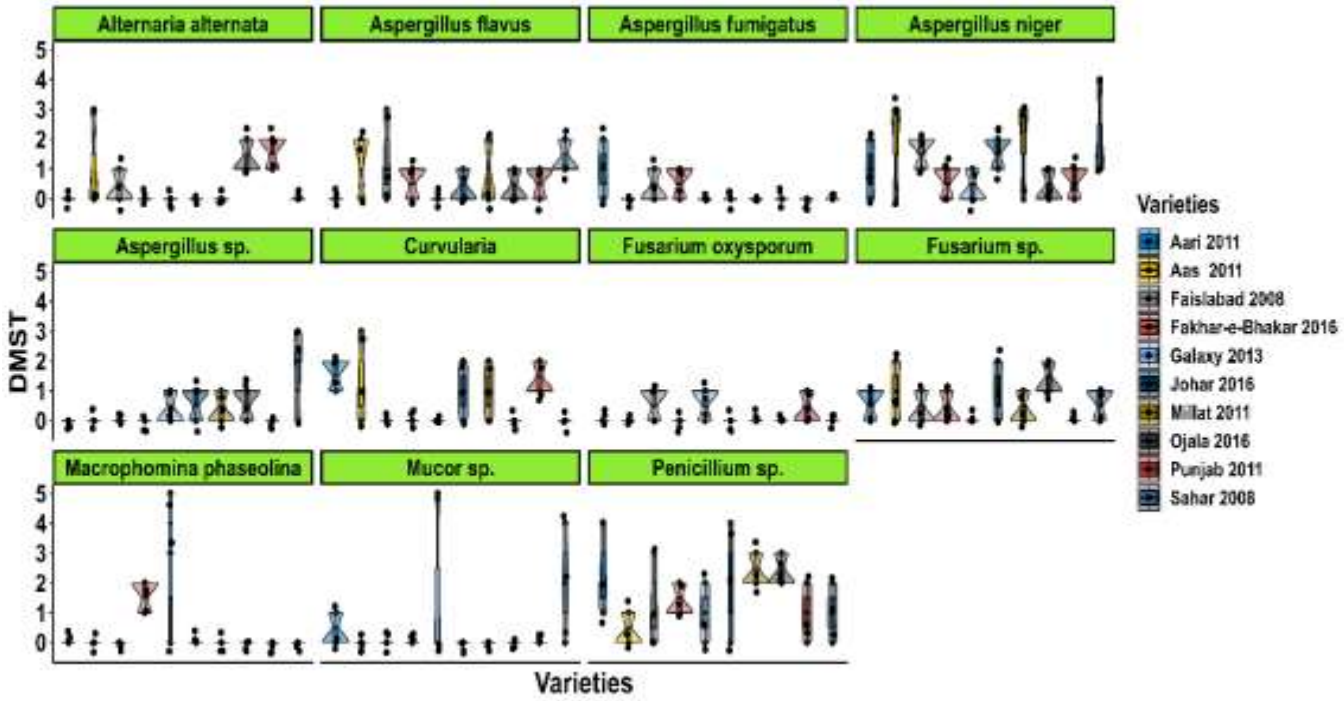


**Figure 3 Distribution** of fungal mycoflora from agar plate method on different wheat genotypes. Y-axis representing DMST = distribution media sterilized method. Galaxy 2013 showing the highest peak with maximum fungal distribution

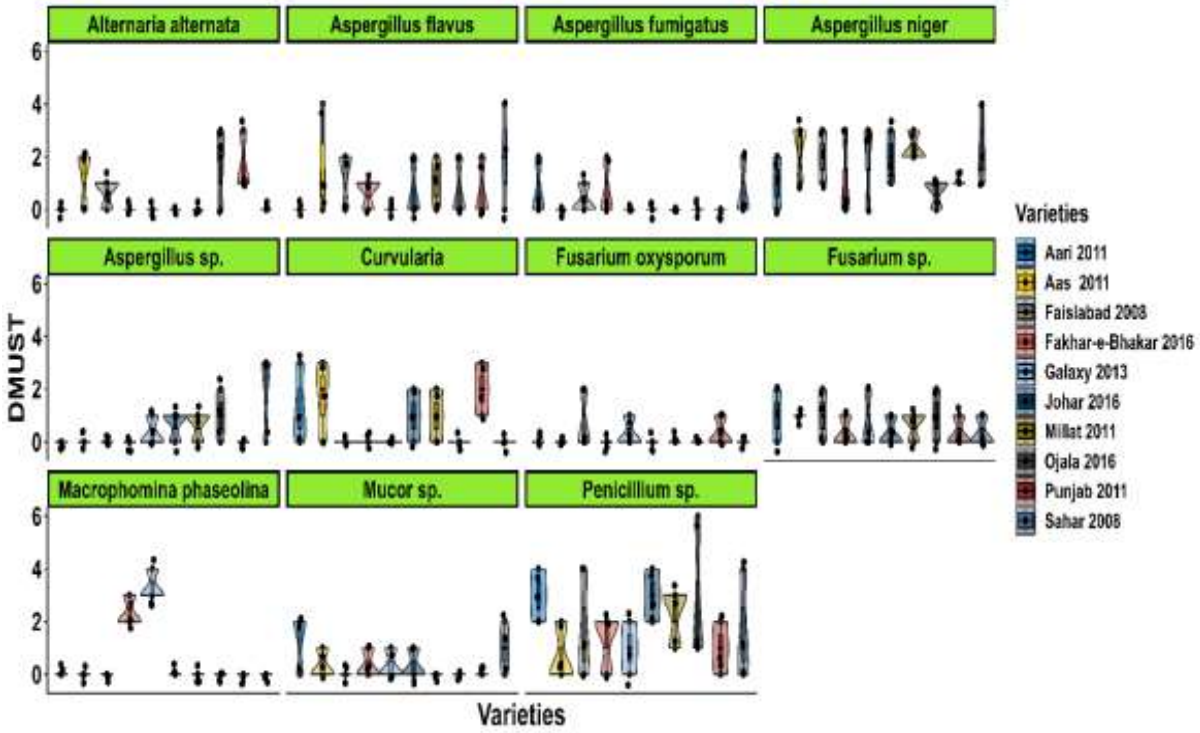


**Figure 4** Distribution of fungal mycoflora from unsterilized agar plate method on different wheat genotypes. Y-axis representing DMUST = distributionmedia Unsterilized method. Ojala 2016 showing the highest peak with maximum fungal distribution

In the sterilized agar plate method, 66 colonies were present and in unsterilized method, there were a total of 79 colonies. In the agar plate method, *Aspergillus niger* had a higher percentage contribution value of 18% and 21% in sterilized and unsterilized treatments, respectively. *Aspergillus fumigatus* had a lower contribution value of 3.03% and 2.59% in sterilized and unsterilized seeds, respectively. Amongall 10 wheat varieties, Sahar 2008 showed the maximum number of colonies, while Fakhar-e-Bhakkar 2016 and Punjab 2011 showed a smaller number of colonies (Figure 3 and Figure 4).

**Relative Frequency of fungal mycoflora**

In all wheat varieties, *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillussp., Alternaria alternata, Curvularia sp., Fusarium oxysporum, Fusarium sp., Penicillium, Macrophomina phaseolina* and *Mucor sp.* had relative frequencies of 40%, 30%, 3%, 13%, 13%, 33%, 3%, and 20% respectively (Figure 5 and Figure 6).

Among all ten wheat varieties, in sterilized agar plate method, *Aspergillus niger, Asperillus flavus, Aspergillus fumigatus, Aspergillus sp., Alternaria alternata, Curvularia sp., Fusarium oxysporum, Fusarium sp., Penicillium, Macrophomina phaseolina* and *Mucor sp.* had relative frequency values of 70%,46%, 16%, 26%, 26%, 40%, 16%, 46%, 76%, 16%
and 13% (Figure 7). In unsterilized agar plate method,it was 83%, 43%, 13%, 30%, 30%, 36%, 10%, 53%, 76%, 20% and 26% respectively (Figure 8).

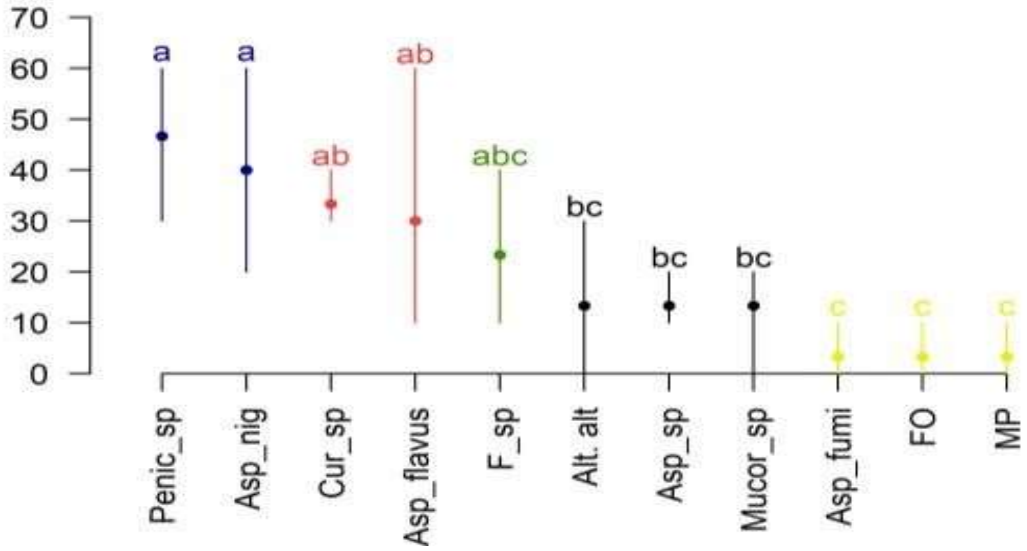


**Figure 5** Relative Frequency of fungal mycoflora of blotter paper sterilized method.Penic_sp = *Penicillium* sp., Asp_nig = *Aspergillus niger*, Cur_sp = *Curvularia* sp., Asp_flavus = *Aspergillus flavus*, F_sp
= *Fusarium* sp., Alt.alt = *Alternaria alternata*, Asp_sp = *Aspergillus* sp.,Mucor_sp = *Mucor* sp., Asp_fumi = *Aspergillus fumigatus*, FO = *Fusariumoxysporum* and M = *Macrophomina*

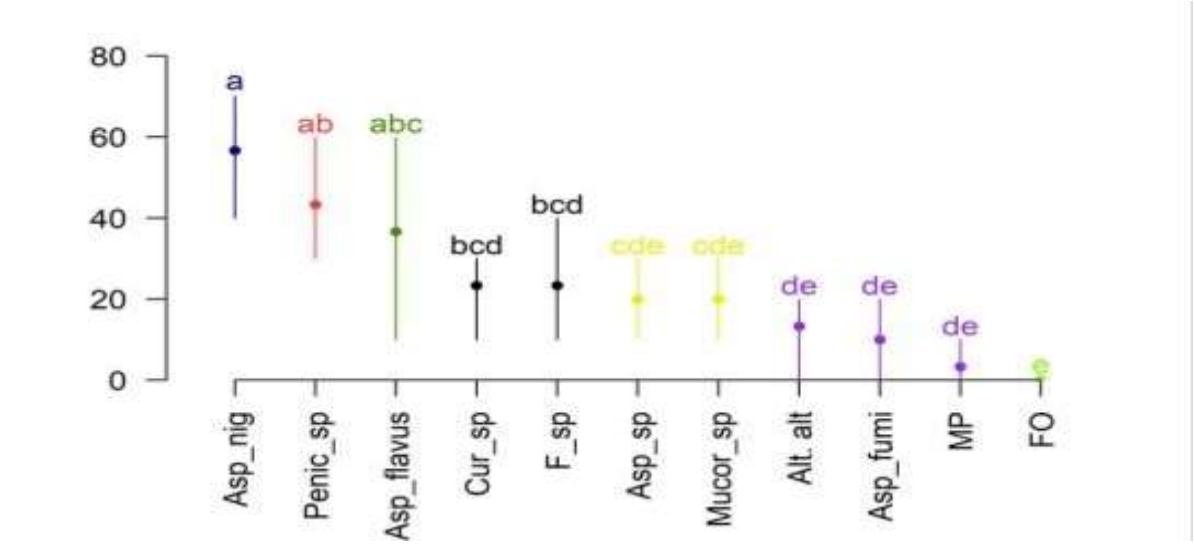


**Figure 6** Relative Frequency of fungal mycoflora of blotter paper unsterilized method.Asp_nig = *Aspergillus niger*, Penic_sp = *Penicillium* sp., Asp_flavus = *Aspergillusflavus*, Cur_sp = *Curvularia* sp., F_sp = *Fusarium* sp., Asp_sp = *Aspergillus* sp., Mucor_sp = *Mucor* sp., Alt.alt = *Alternaria alternata*, Asp_fumi = *Aspergillus fumigatus*, MP = *Macrophomina phaseolina* and FO = *Fusarium oxysporum*

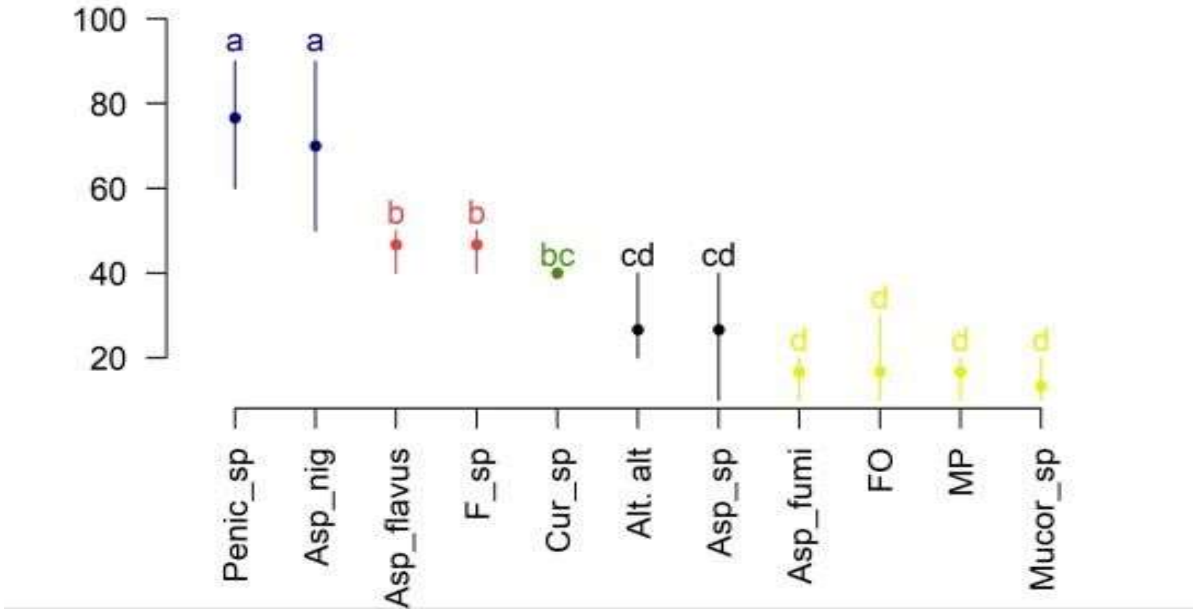


**Figure 7** Relative Frequency of fungal mycoflora of agar plate sterilized method. Penic_sp = *Penicillium* sp., Asp_nig = *Aspergillus niger*, Asp_flavus = *Aspergillus flavus*, F_sp = *Fusarium* sp., Cur_sp = *Curvularia* sp., Alt.alt = *Alternaria alternata*, Asp_sp = *Aspergillus* sp., Asp_fumi = *Aspergillus fumigatus*,FO = *Fusarium oxysporum*, MP = *Macrophomina phaseolina* and Mucor_sp = *Mucor* sp

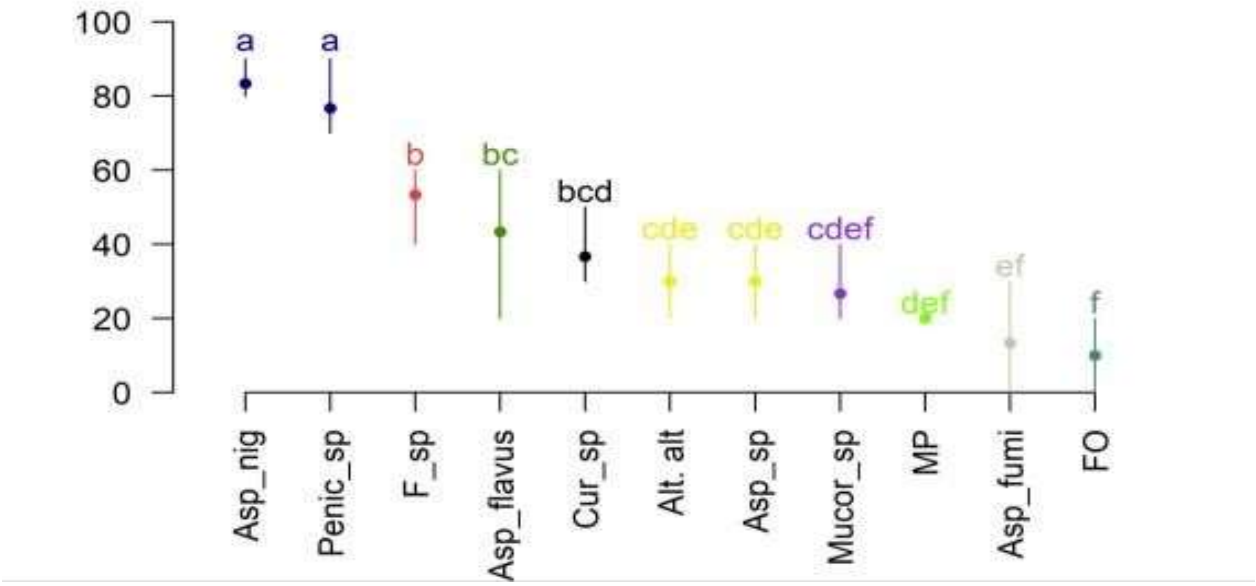


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**Figure 8** Relative Frequency of fungal mycoflora of agar plate unsterilized method.Asp_nig = *Aspergillus niger*, Penic_sp = *Penicillium* sp., F_sp = *Fusarium* sp., Asp_flavus = *Aspergillus flavus*, Cur_sp = *Curvularia* sp., Alt.alt = *Alternaria alternata*, Asp_sp = *Aspergillus* sp.,Mucor_sp = *Mucor* sp., MP = *Macrophomina phaseolina*, Asp_fumi = *Aspergillus fumigatus* and FO = *Fusarium oxysporum*

**Relative Density of Fungal mycoflora**

The results were evaluated for the sterilized seed blotter paper method and the unsterilized blotter papermethod. For the sterilized blotter paper method, *Aspergillus niger* had a higher relative density (33%) in Faisalabad 2008, Punjab 2011, and Fakhar-e-Bhakkar 2016 in all three varieties. *Aspergillus flavus* had more relative density (22%) in Aas 2011. *Alternaria alternata* had a higher relative density (33%), which was present in variety Faisalabad 2008 and Punjab 2011. *Curvularia* with a higher relative density (50%) was present in Johar 2016 and lower (19%) in Millat 2011. *Fusarium oxysporum*, with a high relative density (16%) was present in Galaxy 2013. *Penicillium*'s higher relative density (33%) present in variety Ojala 2016 and Fakhar-e-Bhakkar 2016. *Macrophomina phaseolina* and *Mucor* both hada higher relative density (33%) in Galaxy 2013 (Figure 9). In an unsterilized blotter paper, *Aspergillus niger* had a higher relative density (25%) in Galaxy 2013. *Aspergillus flavus* had higher relative density (33%) inFakhar-e- Bhakkar 2016 and lower one (6%) in Millat2011. *Alternaria alternata* had a higher relative density (50%) value in Faisalabad 2008 and a lower (11%) in Aas 2011. *Fusarium oxysporum* had a relative density of 0% in all varieties. *Penicillium* hada higher relative density (33%) in variety Punjab 2011and lower (12%) in Sahar 2008. *Macrophomina phaseolina* had a higher relative density (16%) inGalaxy 2013. *Mucor* had a higher relative density (30%) in Aari 2011 and a lower (5%) in Aas 2011 (Figure 10).

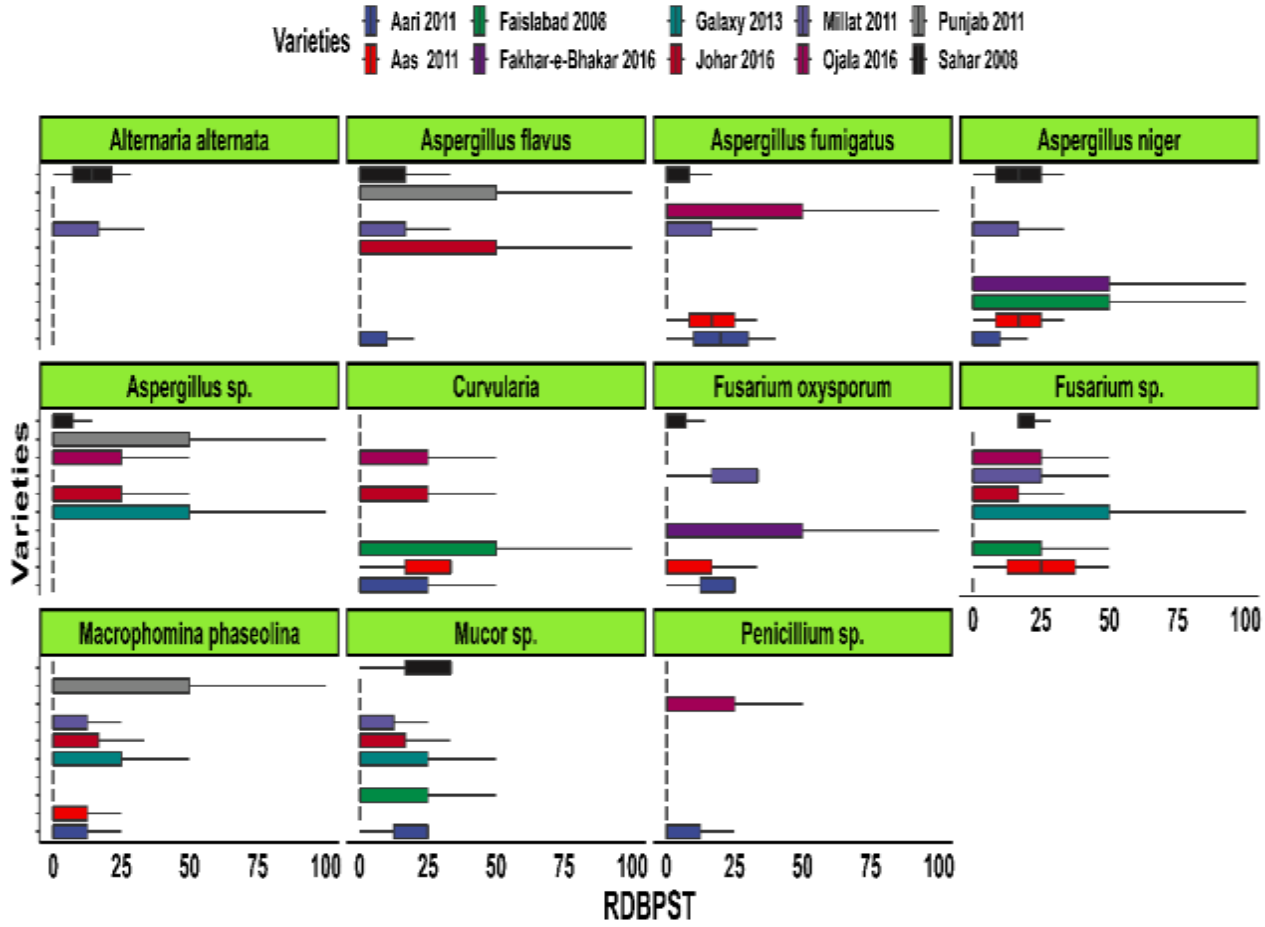


**Figure 9** Relative density of fungal mycoflora from sterilized wheat genotypes through blotter paper method. Fungi including *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus* sp., *Curvularia* sp., *Fusarium oxysporum*, *Fusarium* sp., *Macrophomina phaseolina*, *Mucor* sp., and *Penicillium* sp. were screened on different wheat genotypes

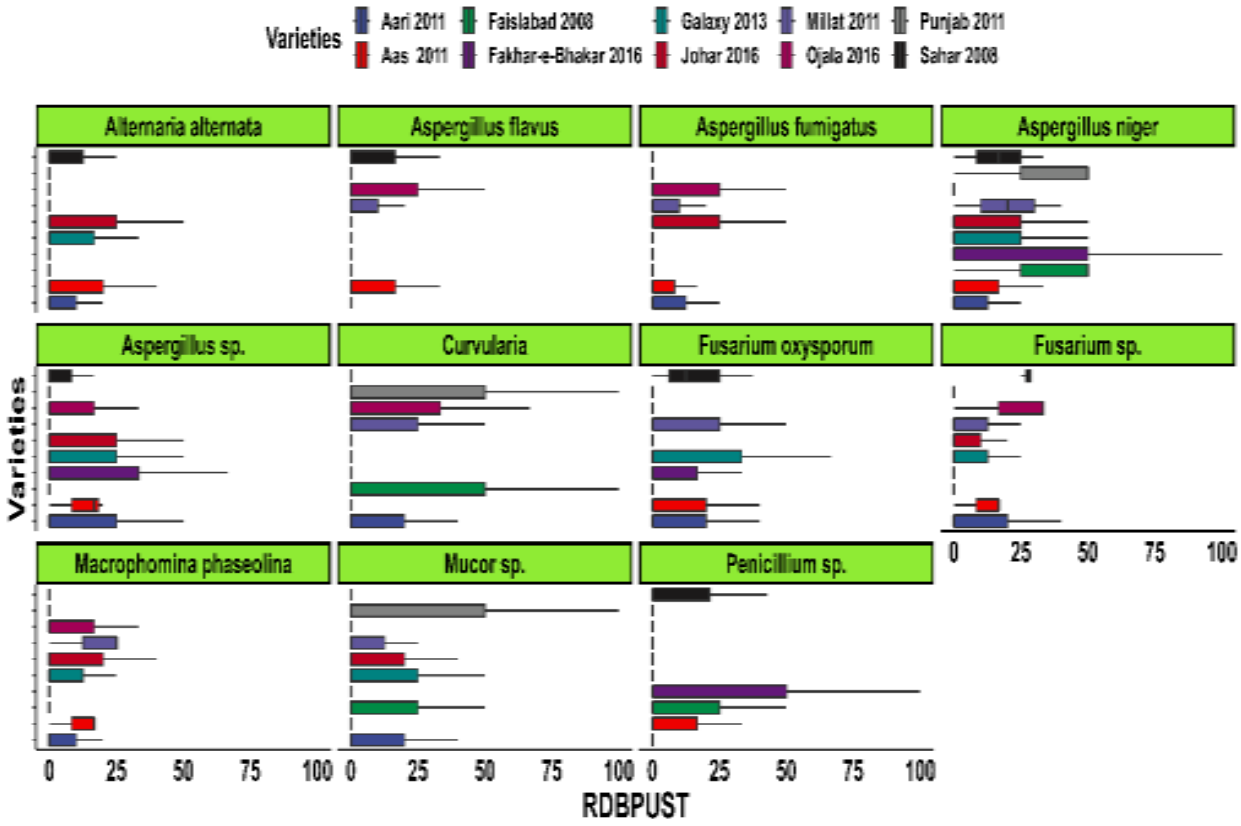


**Figure 10** Relative density of fungal mycoflora fromUnsterilized wheat genotypes through blotter paper method. Fungi including *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*,*Aspergillus* sp., *Curvularia* sp, *Fusariumoxysporum*, *Fusarium* sp.,*Macrophomina phaseolina*, *Mucor* sp., and *Penicillium* sp. were screened on different wheat genotypes

For the sterilized agar plate method, *Aspergillus niger* relative density (30%) was higher in Aas 2011. *Aspergillus flavus* relative density (20%) was higher in Faisalabad 2008 and lower (4%) in Ojala 2016. *Alternaria alternata*, with a higher relative density (31%) present in Punjab 2011. *Fusarium sp*. with a high relative density (20%) present in Ojala 2016. *Penicillium* with a higher relative density (37%) was present in Ojala 2016. *Macrophomina phaseolina* higher relative density (36%) present in Galaxy 2013 and lower (31%) in Fakhar-e- Bhakkar 2016. *Mucor* had a higher relative density (33%) in Galaxy 2013 (Figure 11). For the unsterilized agar plate method, *Aspergillus niger* had a higher relative density (28%) in Millat 2011 and a lower value (7%) in Ojala 2016. *Aspergillus flavus* had a higher relative density (20%) in Sahar 2008. *Alternaria alternata* had a higher relative density (25%) in Ojala 2016. *Penicillium* had a higher relative density (38%) present in Aari 2011 and a lower (6%) present in Aas 2011. *Macrophomina phaseolina* had a higher relative density (41%) present in Galaxy 2013 and a lower value present in Fakhar-e- Bhakkar 2016 (36%) (Figure 12).

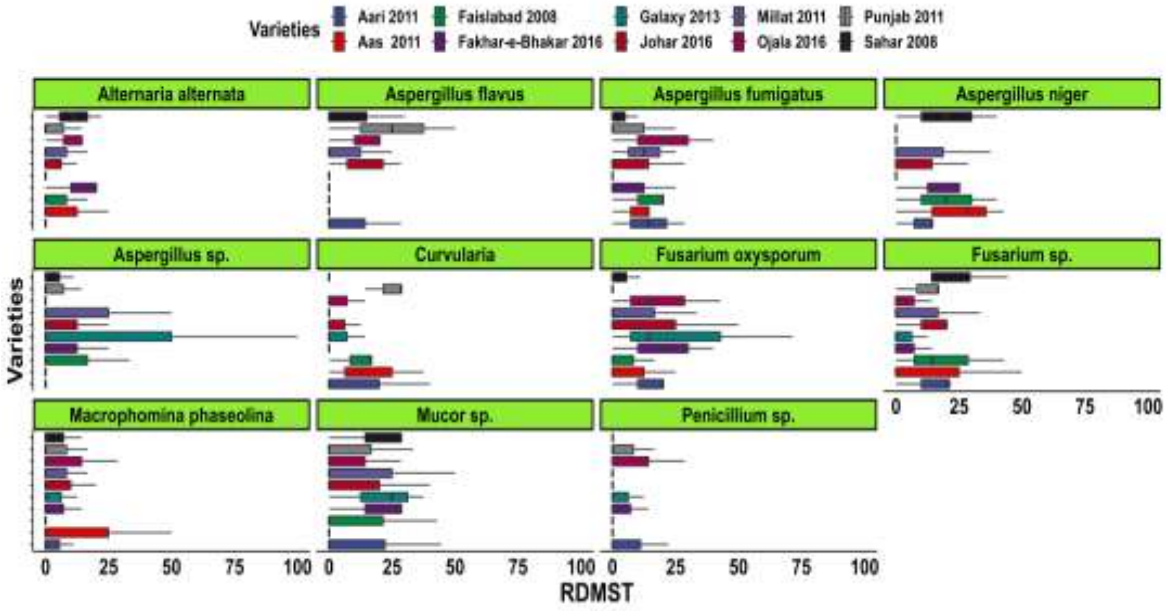


**Figure 11** Relative density of fungal mycoflorafrom sterilized wheat genotypes through agar plate method. Fungi including *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Curvularia* sp., *Fusarium oxysporum*, *Fusarium* sp., *Macrophomina phaseolina*, *Mucor* sp., and *Penicilliumsp.* were screened on different wheat genotypes

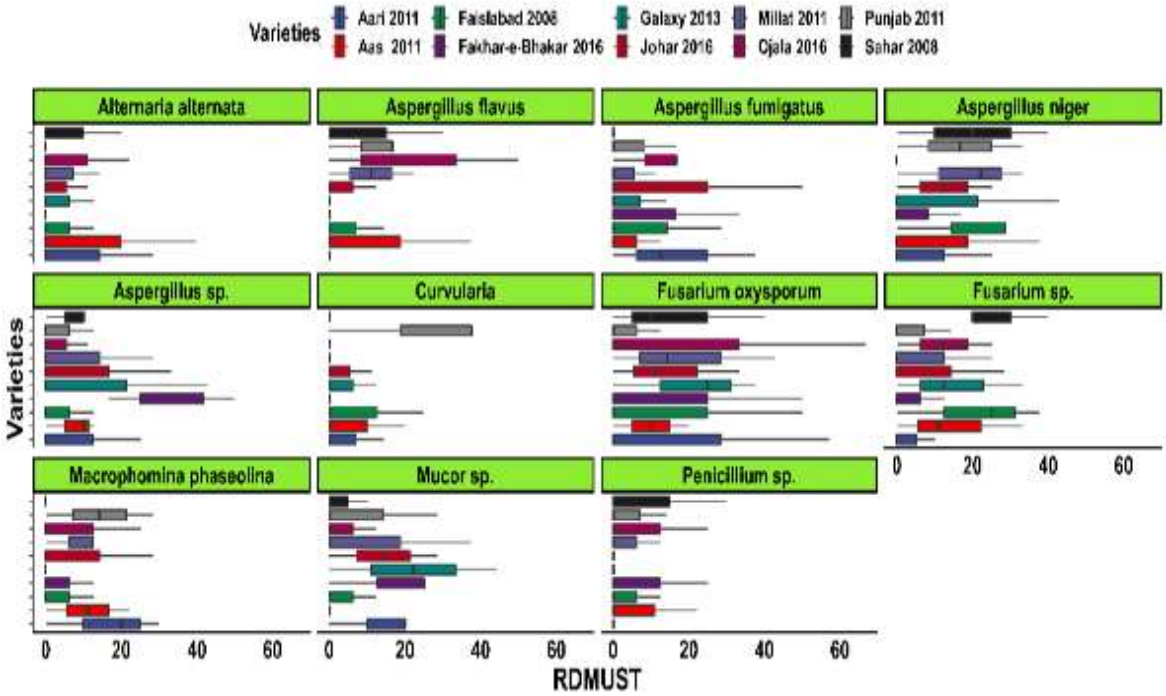


**Figure 12** Relative density of fungal mycoflorafrom unsterilized wheat genotypes through agar plate method. Fungi including *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Curvularia sp.*, *Fusarium oxysporum*, *Fusarium* sp., *Macrophomina phaseolina*, *Mucor* sp., and *Penicilliumsp.* were screened on different wheat genotypes

**Evaluation of Seed Quality Parameters**

In this study, germination test and seed vigour test were conducted and it was found that temperature greatly affects the seedling emergence and root and shoot length of wheat. Growth parameters were also influenced by treatments of inoculated seeds (PGPR1 and PGPR 2) and non-inoculated seeds (control). Of all treatments, 20 °C was the best; 25 °C was the second best, giving the highest germination rate and maximum shoot and root length; and at 15 °C, the lowest rate of seed germination and seed vigour was being observed. Ojala 2016, Johar 2016, Galaxy 2013, Aas 2011, and Punjab 2011 performed maximum in both seed quality parameters, while the rest of the varieties, including Millet 2011, Aari 2011, Fakhar-e-Bhakkar 2016, Sahar 2008 and Faisalabad 2008 had the lowest performance in seed quality tests of all treatments in all three temperature regimes. In seedling vigour, the root length is longer than the shoot. Ojala 2016 was the dominant variety overall in all treatments and temperature regimes, while Sahar 2008 gave poor performance in the rest of the experiment on seed quality parameters. While PGPR

2 treatment give the highest growth in seedling germination and growth parameters regardless of the seed-borne fungi.

**Correlation analysis of seedling vigor test**

The data from the seedling vigour test was analyzed by Pearson's correlation feature and is used to identify differences and relationships between two variables, growth parameters and temperature, under different treatments that may affect the results. In the control treatment, Pearson's correlation results revealed that Root_15C, Root_20C, Root_25C, Shoot_15C, Shoot_20C and Shoot_25C showed highly significant (p-value < 0.001) correlation with one another. Root_15C showed a highly significant (p-value < 0.001) association with all the traits, among which Root_25C and Root_20C showed the highest significant correlations of 0.95 and 0.90, respectively. Root_15C with Shoot_20C showed a strong positive correlation of 0.78 within this association, Sahar 2008 variety showed a strong negative correlation (r = - 1.00***). Root_20C with Shoot_20C and Root_25C showed strong positive correlation values of 0.80 and 0.89, respectively. Root_25 with Shoot_25 showed a highly significant association, with correlation values of 0.80 within this, Millat 2011 (r = - 1.00***) and Ojala 2016 (r = 1.00***) showed strong negative and strong positive correlations, respectively. Shoot_15C with Shoot_25C showed the highest positive correlation value of 0.91 and Shoot_20C with Shoot_25 indicated a strong positive correlation (r = 0.88) (Figure 13).

In the PGPR 1 treatment, Pearson's correlation results indicated that all the parameters had a highly significant (p-value < 0.001) correlation with one another. Shoot_15C showed highest strong positive correlation with Shoot_25C (r = 0.83***). Shoot_20C showed a highly significant association with Root_20C and Shoot_25C, with correlation values of 0.90 and 0.95, respectively. Root_15C showed a highly significant association with Root_20C and Root_25C, with correlation values of

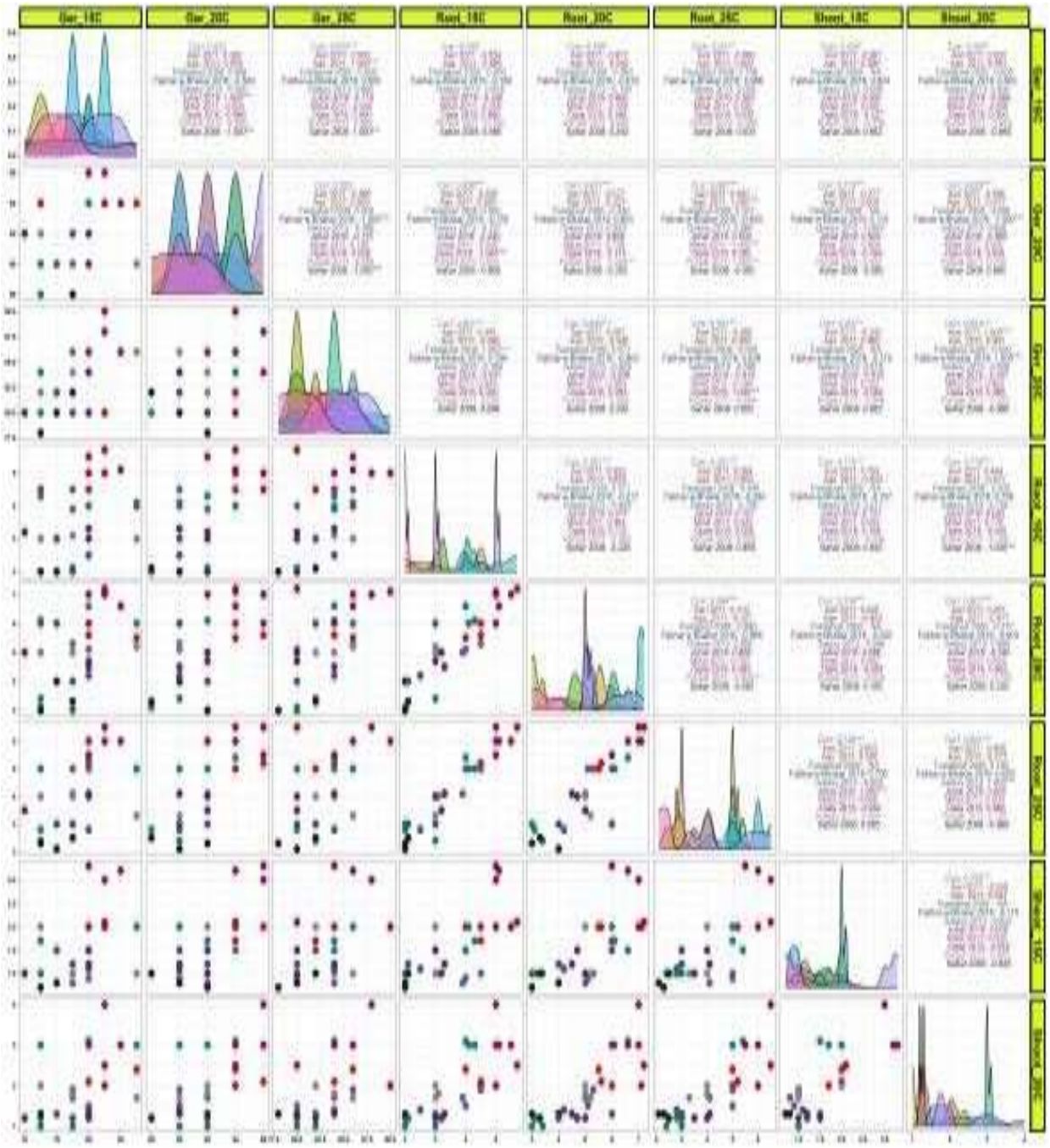

**Figure 13** Pearson's coefficient of correlation for Seedling Vigour Parameters (Shootlength and root length) of 10 wheatgenotypes at three temperature regimes15 °C, 20 °C and 25 °C under control treatment. Color coded alphabets and digits represent 10 wheat genotypes.Variable distribution is presented ondiagonal, upper diagonal signifies correlation coefficient and lower diagonal plots display the distribution of seedling parameters of different wheat genotypes in control condition. Corr = correlation; Root_15C= root length (cm) at 15 °C; Root_20C = root length (cm) at 20 °C; Root_25C = root length (cm) at 25 °C; Shoot_15C = shoot length (cm) at 15 °C; Shoot_20C = shoot length (cm) at 20 °C and Shoot_25C = shoot length (cm) at 25 °C

Ojala 2016 variety showed a strong positive correlation (r = 1.00***) with most of the growth parameter associations at different temperatures in PGPR 1 (Figure 14). PGPR 2 treatment revealed that all parameters had highly significant association (p-value < 0.001) with one another, showing highest values of correlation in comparison to PGPR 1 and control. Shoot_15C showed highest strong positive correlation with Shoot_20C and Shoot_25C, with a value of 0.94in both associations. Shoot_20C showed a strong positive correlation with Shoot_25C (r = 0.95***). Root_15C showed a strong positive correlation with Root_20C (r = 0.94***) and Root_25C (r = 0.96***).

Root_20C had a highly significant association with Shoot_25C (r = 0.90***) and Root_25C (r = 0.96***). Aas 2011 showed a positive correlation in all the associations in PGPR 2 (Figure 15)

**Seed Vigour Test and Seedling Length Vigour Index**

At 15 °C, Ojala 2016 had shoot and root length of 3.67 cm and 7.27 cm in PGPR 2, while the same variety had shoot and root length of 5.67 cm and
8.07 cm in the same treatment at 20°C. Faisalabad 2008 gave a lower value of 3.67 cm in PGPR 2 at 20
°C. At 20°C, in PGPR 1, Ojala 2016 had a root length value of 7.50 cm and Faisalabad 2008 gave a lower value of 3.67 cm. At a temperature of 25 °C, in PGPR2, Ojala 2016 had shoot and root length values of 4.43cm and 7.5 cm, while in PGPR 1, the same variety had shoot and root length values of 4 cm and 6.56 cm, respectively. In control, Ojala 2016 had shoot and rootlength values of 3.2 cm and 6 cm, and Sahar 2008 had shoot and root length of 0.96 cm and 2.3 cm, respectively, at 25 °C.

At 15 °C, PGPR 1 and PGPR 2 had higher SLVI values of 672, 764, and 930, respectively, in Ojala 2016, while PGPR 1 and PGPR 2 had lower SLVI values of 218, 353, and 400, respectively, in Sahar 2008. At 20 °C, in Ojala 2016, treatment control,PGPR 1 and PGPR 2 had higher values of SLVI that were 940, 1177 and 1373, however lower value observed in Faisalabad 2008 in its treatment control, PGPR 1 and PGPR 2, which were 380, 460 and 556, respectively. Ojala 2016 at 25 °C, in control, PGPR 1and PGPR 2 had higher values of SLVI that were 792,936 and 1074, respectively, while a lower value was observed in Sahar 2008 in its treatment of control, having a value of 261. Lower values observed inFaisalabad 2008 in its treatment of PGPR 1 and PGPR
2 values of SLVI were 317 and 394, respectively (Figure 16, Figure 17 and Figure 18).

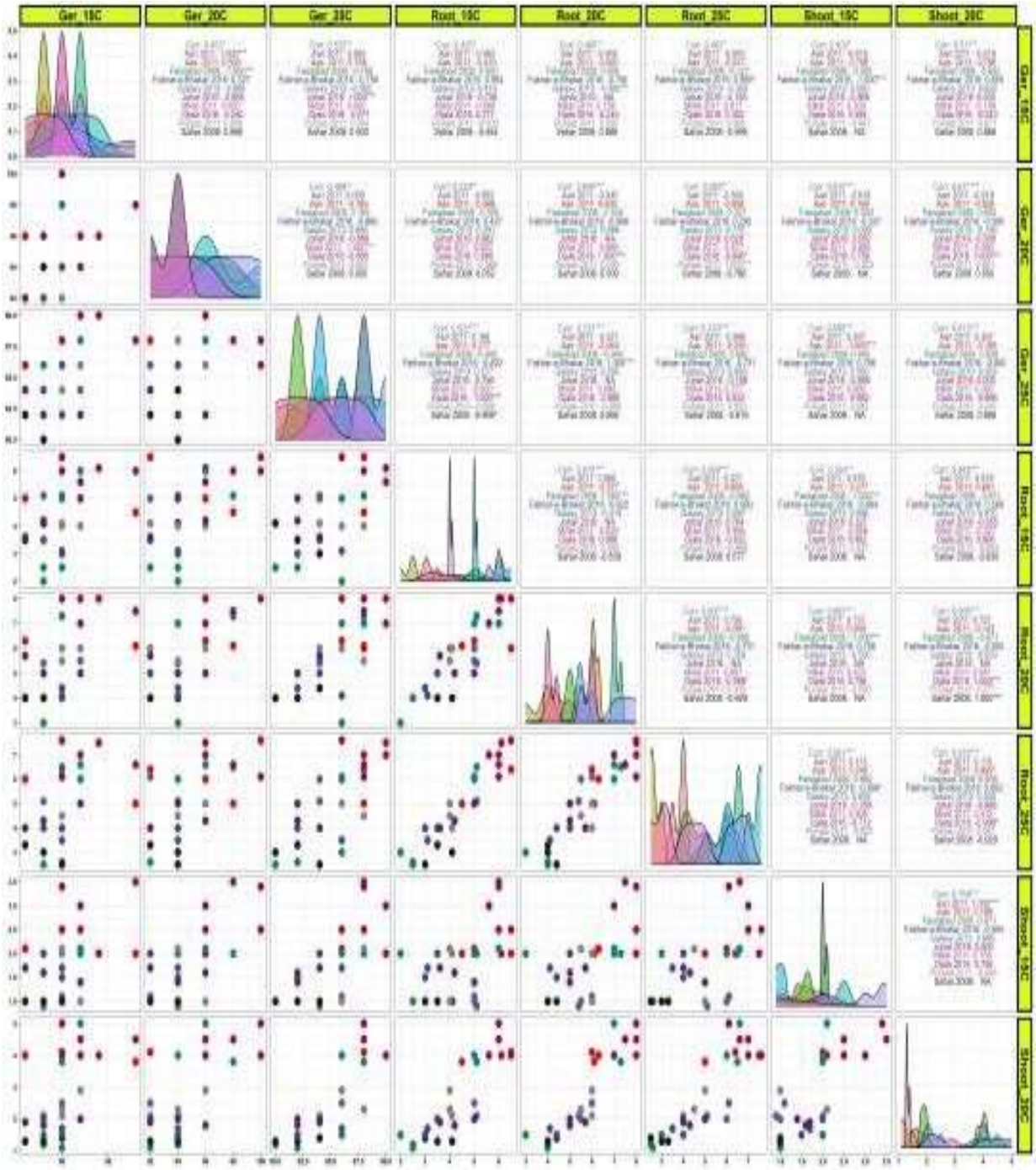


**Figure 14** Pearson's coefficient of correlation for Seedling Vigour Parameters (Shootlength and root length) of 10 wheatgenotypes at three temperature regimes15 °C, 20 °C and 25 °C under PGPR 1 treatment. Color coded alphabets and digits represent 10 wheat genotypes.Variable distribution is presented ondiagonal, upper diagonal signifies correlation coefficient and lower diagonal plots display the distribution of seedling parameters of different wheat genotypes in PGPR 1 condition. Corr = correlation; Root_15C= root length (cm) at 15 °C; Root_20C = root length (cm) at 20 °C; Root_25C = root length (cm) at 25 °C; Shoot_15C = shoot length (cm) at 15 °C; Shoot_20C
= shoot length (cm) at 20 °C and Shoot_25C = shoot length (cm) at 25 °C

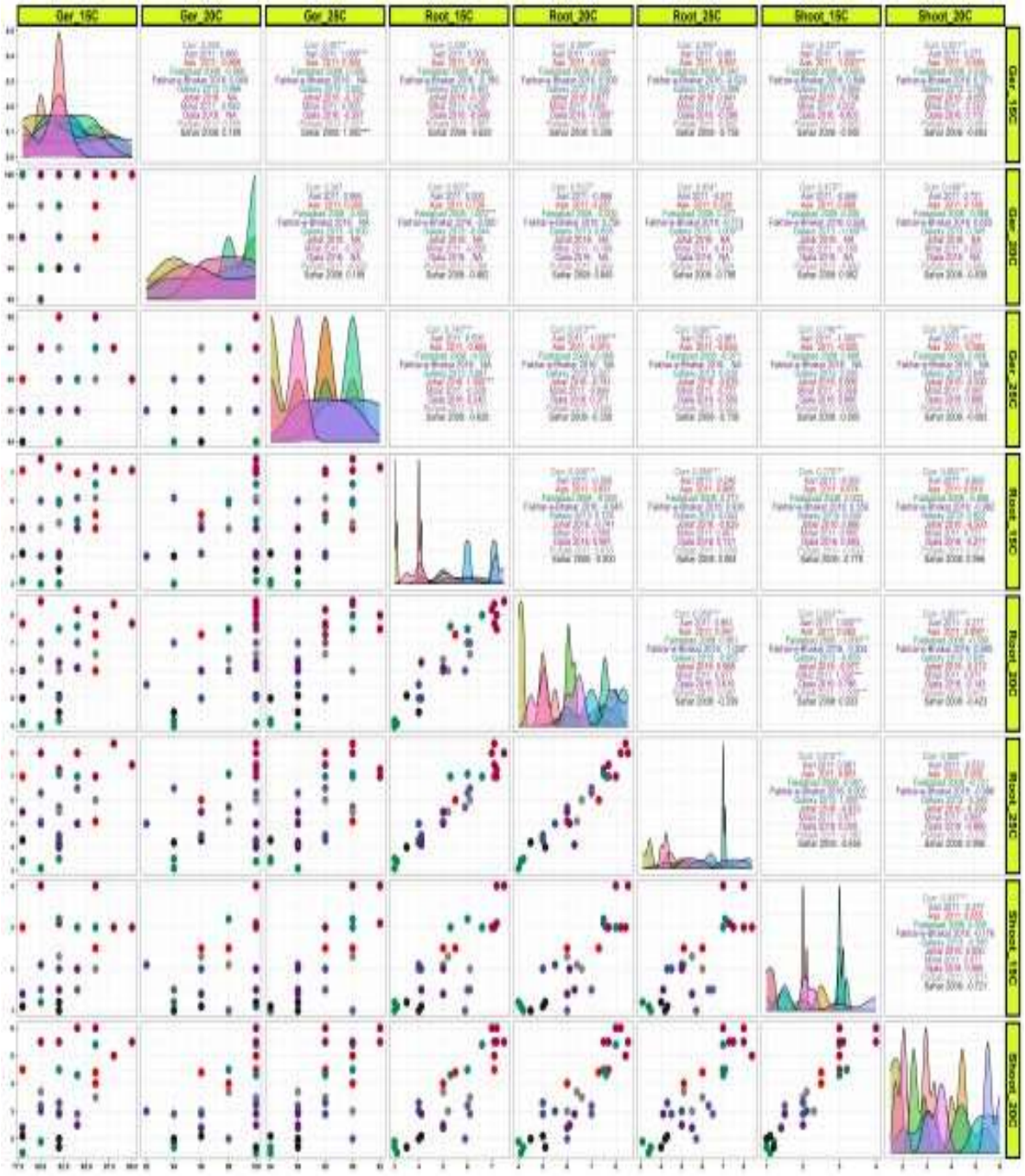


**Figure 15** Pearson's coefficient of correlation for Seedling Vigour Parameters (Shoot length and root length) of 10 wheat genotypes at three temperature regimes 15 °C, 20 °C and 25 °C under PGPR 2 treatment. Color coded alphabets and digitsrepresent 10 wheat genotypes at three temperature regimes 15 °C, 20 °C and 25 °C under PGPR 1 treatment. Color coded alphabets and digits represent 10 wheat genotypes. Variable distribution ispresented on diagonal, upper diagonal signifies correlation coefficient and lower diagonal plots display the distribution of seedling parameters ofdifferent wheat genotypes in PGPR 2condition. Corr = correlation; Root_15C= root length (cm) at 15 °C; Root_20C = root length (cm) at 20 °C; Root_25C = root length (cm) at 25 °C; Shoot_15C = shoot length (cm) at 15 °C;Shoot_20C = shoot length (cm) at 20 °Cand Shoot_25C = shoot length (cm) at 25°C

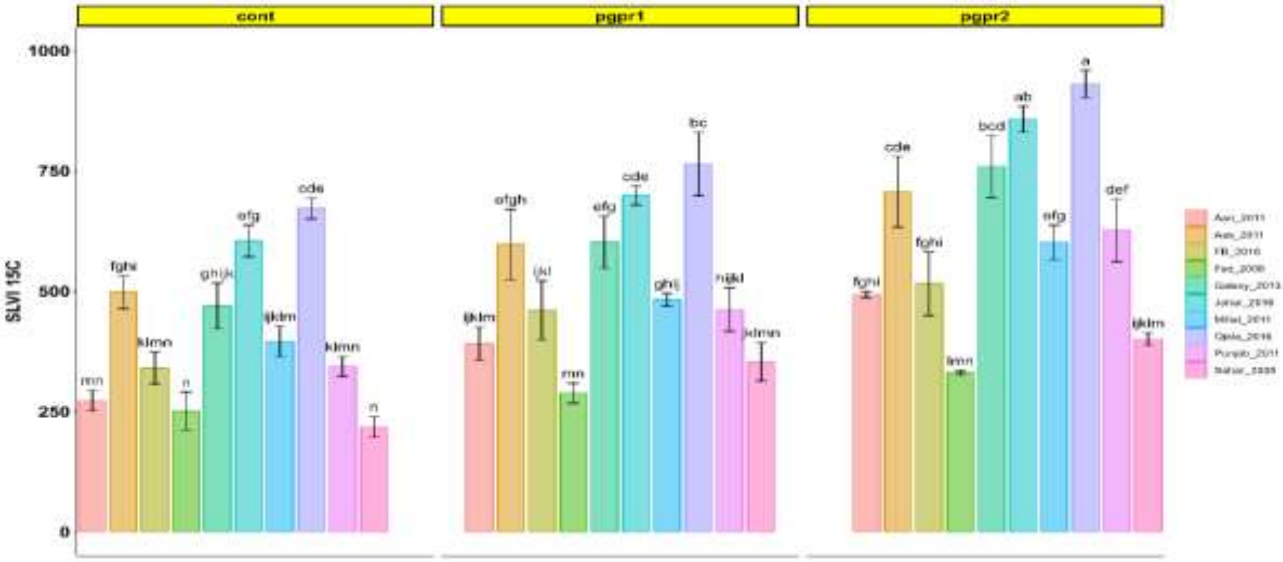


**Figure 16** Seedling Length Vigour Index of 10 wheat genotypes at 15 °C under control,PGPR 1 and PGPR 2 treatment. Different letters indicate significant differences among treatments based on LSD test at P < 0.05

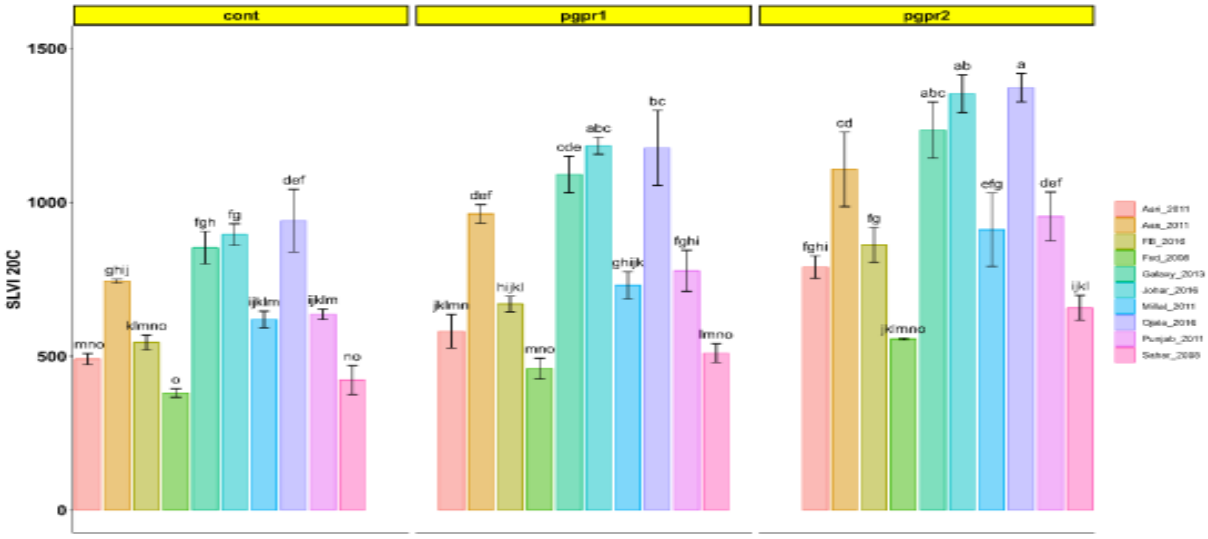


**Figure 17** Seedling Length Vigour Index of 10 wheat genotypes at 20 °C under control,PGPR 1 and PGPR 2 treatment. Different letters indicate significant differences among treatments based on LSD test at P < 0.05

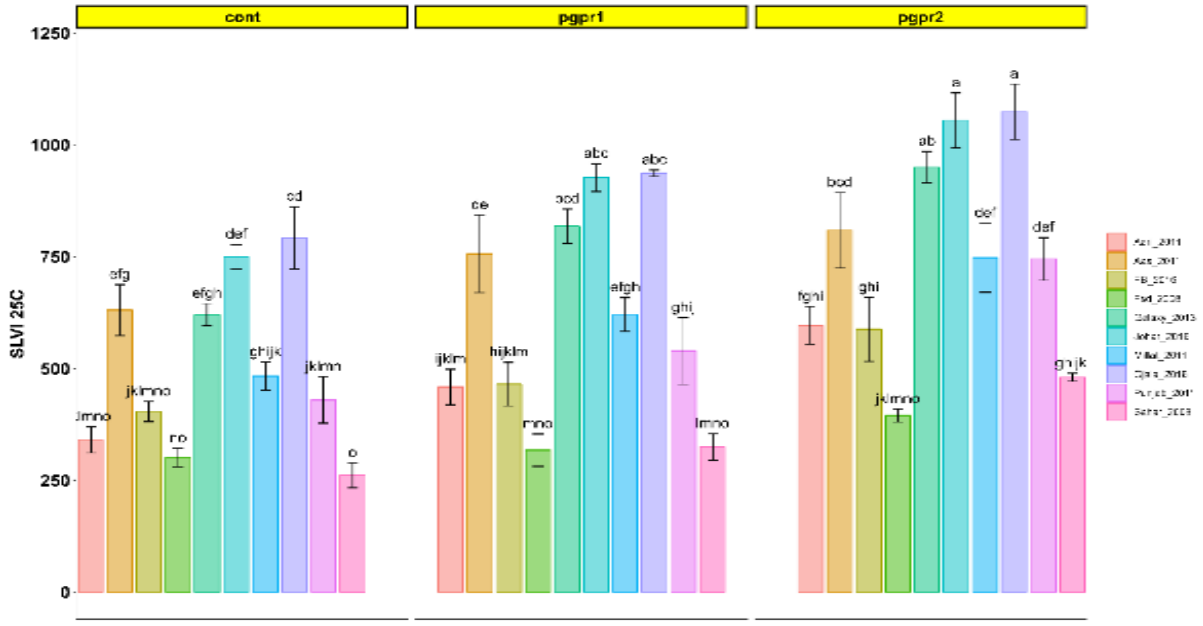


**Figure 18** Seedling Length Vigour Index of 10wheat genotypes at 25 °C under control, PGPR 1 and PGPR 2 treatment. Different letters indicate significant differences among treatments based on LSD test at P < 0.05

**Discussion**

This study was conducted to evaluate the efficacy of a few priming procedures on wheat cultivar germination, seedling development and seed-borne fungi in response to the Plant Growth Promoting Rhizobacteria (PGPR) inoculation. Seed priming speeds up the germination process and improves the seedling emergence rate (Devika et al., 2021). Biopriming depends on the employment of advantageous microorganisms, i.e., PGPR, that encourage plant development in seed preparation. Plant growth enhancement and plant pathogen growth suppression have both been attributed to PGPRs (Mitra et al., 2021). There are a number of ways that bacteria can both increase plant growth (by producingphytohormones and nitrogen fixation) and decrease plant disease (by competing for nutrients, space, and ecological niches, producing hydrocyanic acid, siderophores, hydrolases, and antifungal compounds, or by induced systemic resistance (ISR)) (Wang et al.,2018). Varying temperature and PGPR seed treatment simultaneously affect the germination rate and seedling vigour and also have an effect on seed-bornepathogens. Seedling phase of plant plays a significantrole in better plant growth under environmental stresses, as clarified in preceding studies (Ali et al., 2022; Khan et al., 2020; Shah et al., 2021).

In the present research work, a few seed priming techniques and different quantitative estimation fungi on seeds were discussed. Fungi infected seeds were studied by observing the frequency, distribution and density of isolates. Ten different wheat cultivars were utilized to analyze the pattern of diversity among fungi. Valued data was recovered regarding fungi seed-borne and surface contamination. By using this practice, it is appropriateto get the resistant wheat varieties data. The molds (*Aspergillus flavus* and *A. niger*) werethe most predominant external mycoflora, these results agreed with those reported previously (Barros et al., 2005). Bashir et al., (2012) who stated that the high degree of mold contamination in stored grains and animal feeds is a measure of their quality assurance. The fungal incidence was maximum in unsterilized seeds than sterilized seeds in both the blotter paper andagar plate methods. In all ten wheat varieties, Sahar 2008 and Aas 2011 show the maximum fungal incidence, and Punjab 2011 and Fakhar-e-Bhakkar 2016 show less fungal incidence in both methods and their treatments of sterilized and unsterilized seeds. All isolates that were observed morphologically fall into two genera, *Fusarium* and *Alternaria* (Khaledi etal., 2021).

A similar study was conducted in which he notedthe maximum incidence of *Aspergillus niger* 21% in agar plate and 17% in blotter paper method. *Aspergillus niger* and *Penicillium sp.* show a higher distribution and relative density in the agar plate method than the in blotter paper method (Habib et al., 2011). Hajihasani et al., (2012) reported 15 fungal species isolated from three wheat varieties in Iran. Most among them were *A. niger, A. flavus*, *Alternariaalternata*, *Curvularia* sp. and *Penicillium* sp. (Shehzadi et al., 2016). Tahira et al., (2018) reported the highest percentage incidence of *Fusarium moniliforme* among the wheat cultivars of Sindh. *Aspergillus* and *Penicillium* had a higher percentage contribution to causing the infection in wheat seeds. Gaddeyya et al., (2012) investigated that *Aspergillus niger, A. fumigatus* and *A. flavus* had more %age contribution in six crops of sugarcane, red gram paddy, corn, ragi and cotton. A Tunisian early study showed, related results regarding *Alternaria* genusdominance in grains of wheat (Belkacem-Hanfi et al., 2013). A significant

number of fungi were isolated, majorly comprises of *A. flavus* from Portuguese almonds (Rodrigues et al., 2012). *Fusarium* species of *F. equiseti* and *F. acuminatum* isolated from wheat and barley from Spain (Castellá and Cabañes, 2014; Marin et al., 2015; Marín et al., 2012).

Tahira et al., (2018) using the blotter paper technique, isolated five fungal species from wheat seeds. *Fusarium* species mainly *Fusarium incarnatum* domination in Egyptian and Tunisian sorghum was reported earlier (Lahouar et al., 2015). In the Central Anatolia region of Turkey, the main fungal pathogens,including *F. solani*, *F. oxysporum* f.sp. *cumini*, *F.equiseti* and *A. alternata*, were found associated withthe cumin plant (Özer and Bayraktar, 2015). Janmajayet al., (2011) recorded 16 species of fungus in two cultivars of wheat seeds and found that the blotter paper technique was better than the agar plate technique regarding isolation of mycoflora and that seed borne mycoflora was affiliated with the contamination of seeds. It was reported that rice seeds of different varieties were associated with *Alternaria* sp., *Helminthosporium* sp., *F. moniliforme* and *Curvularia* specie (Butt et al., 2011). Kesho and Abebe, (2021) isolated five fungal species from wheatseeds. They also found *A. flavus* to be the most dominant fungus in all different seeds classes of nine crops.

Zafar et al., (2014) reported that the dominant fungi regarding frequency of occurrence were *Aspergillus flavus* (18%) and *Penicillium* sp. (16%), while *A. fumigatus* had only 1% frequency in wheat seeds. While Tahira et al., (2018) studied higher frequency of *Alternaria alternata* 49%, *Aspergillus niger* 46% and *Fusarium* species 42% in seeds of wheat. In our study, we isolated 11 fungi, including *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus sp, Alternaria alternata, Curvularia sp., Fusarium oxysporum, Fusarium sp , Penicillium, Macrophomina phaseolina* and *Mucorsp.* from wheat seeds. Kadege and Lyimo, (2015)isolated 10 fungal species from wheat seeds, with varying frequencies of each fungus. Hussain et al., (2013) reported five fungi from wheat seeds that were *Penicillium* sp. *Alternaria alternata*, *A. flavus*, *A. niger* and *F. moniliform* with %age frequency of 12, 15.2, 17.8, 19 and 36 respectively. Adhikari et al., (2015) from four different locations of Nepal, reported highest percentage frequency of *Alternaria alternata* in wheat seeds. Jedidi et al., (2018) reported higher relative frequency percentage of *Aspergillus* in wheat and barley that were 63% and 70% while *Fusarium* occur in lower %age of 18.18% and 50% in same crops. Jedidi et al., (2018) reported highest density of *Aspergillus* in maize samples and highest relative density of *Alternaria*, *Penicillium*, *Aspergillus* and*Eurotium* in wheat and barley. Jedidi et al., (2017) indicated more prevalence of *A. flavus* in cereals. *Aspergillus niger*, *Alternaria alternata*, *Penicillium* showed higher relative density. Relevant study conducted by Dawood and Elshamry, (2015) in whichhe found *Aspergillus niger* and *A. flavus* to be the mostabundant fungal species in stored grains. Bashir et al.,(2012) isolated five fungal species from local and improved wheat varieties and *Fusarium oxysporum* was found to be relatively abundant. Khaledi et al., (2021) isolated four species of fungi from seeds of Iranian cumin and *Alternaria alternata* found to have highest relative density of 77.8%. Seed-borne fungi pose a major threat to the overall seed health of wheat grains. In our study, seeds from the same set of varieties from which seed borne fungi were isolated were used and subjected to the PGPR's inoculation to assess

the response of seed-borne fungi towards the PGPR inoculated seeds. Among all seed treatments on wheat, PGPR 2 ($N_2$+P) significantly increased seed vigour and germination, followed by PGPR 1 ($N_2$) while in the control treatment lowest seedling emergence and seed vigor were observed, further supported by the study of Babu et al., (2015) on tomato plants. Comparable to our findings, (Dobbelaere et al., 2002)analyzed the maximum development and dry weight of root in PGPR inoculated plants as compared to the control plants. Temperature of 20°C and 25°C were optimum temperature range for wheat in Pakistan (Buriro et al., 2011; Rodriguez et al., 2015). Ojala 2016 was the dominant variety overall in all treatments and temperature regimes in the rest of the experiment of seed quality parameters. In seedling vigor, root length is greater than the shoot. Cassán and Diaz- Zorita, (2016) reported more root length of plant than shoot length in PGPR experimentation. PGPR 2 treatment give the highest growth of seedling germination and growth parameters. Hassan et al.,(2021) reported progressive impact on the plant parameters of sunflower by increasing shoot and root length by PGPR 2 as similar to our findings. Some of the beneficial effects of PGPR treatment including enhanced root growth, were described earlier by Glick,(2012) and Ahemad and Kibret, (2014). Higher shoot and root development in PGPR 1 inoculated plants andseeds had been reported (Karimi et al., 2021; Pankievicz et al., 2015; Xue et al., 2014). Crops understress inoculated with PGPR 1 enhance the surface area, length and volume of roots as compared to the control plants (Caires et al., 2021). Galindo et al., (2021) reported that maize and wheat inoculated with PGPR 1 have more foliage. The results were analyzed by Pearson's correlation feature and was used to identify differences and relationships between two variables growth parameters and temperature under different treatmentsthat may affect the results, as validated Bouteillé et al., (2012). For each of the two variables (root and shoot length at different temperatures), fluctuations were observed between wheat varieties, with treatments showing significant correlations (Figure 1, Figure 2 and Figure 3). Amorim et al., (2018) also demonstrated results of tomato growth parameters based on Pearson correlation analysis similar to our outcomes of correlation analysis. Pedrosa et al., (2020) reported positive results after inoculating maize seeds with PGPR 1 as compared to a control. Bishaw et al., (2013) determined wheat seed health using correlationanalysis. Menard et al., (2021), using all traits means, calculated Pearson's correlation coefficients similar toour analysis. Argaw, (2012), previously studied dual inoculation of PGPR on soyabean increase plant height. Awan et al., (2018) also used correlation analysis to determine significant differences between physiological and biochemical attributes. Uzair et al., (2022) also conducted results using Pearson's coefficient correlation and found significant difference in the growth parameters of wheat. Associative degreefound between disease powdery mildew

rating of severity and selected biochemical constituent using Pearson's correlation coefficient (Aly et al., 2012).

**Conclusion**

The findings demonstrated that, in both the blotter paper and agar plate methods, Sahar 2008, Aas2011, and Aari 2011 displayed the highest fungal incidence out of all 10 wheat varieties. The lowest fungal incidence was found in Fakhar-e-Bhakkar (2016), Punjab (2011), and Faisalabad (2008). *Aspergillus niger* had a greater relative frequency and*Macrophomina phaseolina* had a lower relative frequency in all wheat cultivars. Inoculated treatment (PGPR1 and PGPR2) on seeds also had an impact on growth attributes in comparison to non-inoculated (control). The optimal temperature for all treatments was 20 °C, which provided the highest shoot and rootlength as well as the germination rate. Sahar 2008 didpoorly in all of the other seed quality metrics of the trial, while Ojala 2016 did best in terms of seed vigourand germination across all treatments and temperatureregimes. Regardless of seed-borne fungus, the PGPR 2 treatment resulted in the greatest increase in seedlinggermination and growth metrics.

**Acknowledgement:** Our Acknowledgement is due towards First Fungal Culture Bank of Pakistan, Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, for providing us best facilities for identification of fungal pathogens.

**Conflict of Interest:** Authors have no conflict of interest.

**Funding Information:**

The project was supported by the University of the Punjab.

**Author's Contribution:**

Shama Sharif: Writing and editing of manuscript, Methodology; Zill-e-Huma Aftab: supervisor ; Tehmina Anjum: Supervisor ;Waheed Akram: Data Analysis; Maroof Siddiq: writing and editing of manuscript, data analysis

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