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Vigor and stand establishment of harvested wheat seeds affected by nitrogen fixing microbes with nitrogen ratios

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An experiment was conducted at Agronomy laboratory, The University of Agriculture, Peshawar in summer 2015. To study the effect of different nitrogen fixing microbes with nitrogen ratio on vigor and stand establishment of harvested wheat seeds. Two wheat varieties i.e. Pirsabak-2013 and Shahkar 2013 were sown in Agronomy Research Farm of The University of Agriculture Peshawar. Wheat seeds were pretreated with nitrogen fixing microbes (*Azospirillum* and *Azotobacter*) and various nitrogen ratios (25%, 50%, 75% and 100%) were applied to the seed bed. After harvesting wheat seeds in June, 2015 were subjected to different vigor and viability tests. Complete Randomize Design was used with three replications. Nitrogen fixing microbes with nitrogen ratios increased water imbibitions (89.1%), growth rate (90.1%), germination (88.4%), seed vigor index (89.2%), mean germination time (88.4%), shoot dry weight (89.4%), root dry weight (90.7%), seedling dry weight (89.9%), shoot length (88.6%),seedling length (88.3%), relative water content (92.2%), root/shoot dry weight ratio (89.9%) as compared to control wheat varieties. *Azotobacter* with 50% nitrogen showed highest germination percentage (95.7%), growth rate (13) and mean germination time (6.6). *Azospirillium* with 25 % nitrogen increased seed vigor index (340.7), mean germination time (6.6), longest shoots (3.3cm), lengthy roots (3.6cm), seedling length (6.9cm), shoot dry weight (1g), root dry weight (1.7g) and seedling dry weight (3.6g). Alone nitrogen and alone nitrogen fixing microbes did not increased vigor and viability of wheat varieties. Maximum vigor and viability were observed in Pirsabak-2013 as compared to Shakar-2013. It is concluded that *Azospirillum* with 25% nitrogen increased vigor and stand establishment in Pirsabak-2013 wheat variety.

Keywords: *Triticum aestivum;* nitrogen fixing microbes; nitrogen ratios; vigor; stand establishment

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a member of family Poaceae (Gramineae). It is a long day, annual, self-pollinated and Rabi season crop. Wheat is one of the most important cereal grain crop grown in the worldwide and is a staple food of about one third of the world's population (Khan et al*.,* 2014). Wheat is the main staple food of the people of Pakistan and the country's most important strategic agricultural commodity. It contributes 39.99% to the value added of major agriculture crops. Statistical data of Pakistan during 2012-13 showed that wheat was cultivated on an area of 8660.2 thousand hectares with the total production of 24211.4 thousand tones having yield of 2796 kg ha^{-1}. In Khyber Pakhtunkhwa, wheat was grown on 727.3 thousand hectares with the production of 1257.6 thousand tones having yield of 1729 kg ha^{-1}(NFS and R, 2013-2014).

Bio-fertilizers are product containing living cells of different types of microorganism. Bifertilizer plays a very important role in improving soil fertility by fixing atmospheric nitrogen. In bio-fertilizer the beneficial microorganism speed up and protect plant from pests and diseases and also enhance plant growth. Influence of bio-fertilizers on different seed quality parameter is highly required for future use of bio-fertilizers in seed production technology (Mirshekari and Baser 2010). Changes of food habit of the people and alarming exploded population have led to an increase demand of food consumption. The main role played by nitrogen in improving the yield potentials of newly developed varieties of crop plants cannot be underestimated in sustainable agriculture. The newer increasing cost of nitrogen fertilizer often prevents the use of these major inputs required for crop plants in developing countries. Rout et al. (2001) conducted study during Kharif season, to assess the response of bio-fertilizers on nitrogen economy and maize yield. Bio-fertilizers have beneficial effect on yield. The beneficial effects were higher than the bio-fertilizer cost. A pot experiment was conducted by Mudenoor et al., (2007), to study the effects of seed treatment of micro nutrients supplemented *Azospirillum* bio-fertilizer on dry mater and yield of maize at Karnataka and result indicated high shoot and root dry matter with seed treatment of *Azospirillum*. In this context microbial inoculants offered the most promising supplement if not alternative to chemical fertilizers. Moreover, microbial inoculant does not pose environmental hazards and maintain soil health. An *Azospirillum* bacterial fertilizer assimilates atmospheric nitrogen and fixes in soil and also secrets phytohormones in plant root regions which in turn enhance root growth. Invigoration of low vigor seeds to maintain vigor, viability and productivity in rice may prove beneficial (Basu and Paul, 1979; Pal and Base, 1988). Greef et al., 1999 reported that improvement in DM yield in corn occurred due to higher uptake of Nitrogen from the deeper soil layer. The breadth of the area per leaf profile reduced under high soil nitrogen level (Oscar and Tollenaar 2006). Nitrogen uptake by the corn was increased at higher dose of N application which resulted in increased dry matter (DM) and grain yield which shows a direct relationship between the yield and nitrogen application (Jokela and Randall 1989).

Nitrogen plays a very vital role in the process of grain filling increase leaf area of the crop and may result in increased dry matter production by intercepting more sun light (Wilhelm, 1998). The studies on the effects of these microorganisms on seed vigor and viability are very rare (Alahdadi et al., 2009). The objective of this experiment was to explore the role of sole nitrogen fixing microbes or with nitrogen ratios on wheat seeds vigor and viability.

MATERIALS AND METHODS

Experiment material and Design

Experiment was conducted at Agronomy laboratory, Department of Agronomy, Faculty of Crop Production, The University of Agriculture Peshawar. The experiment was laid out in complete randomize design (CRD) with three replications to study the effects of nitrogen fixing microbes with nitrogen ratios on vigor and viability of harvested wheat seeds i.e. Pirsabak-2013 and Shahkar-2013 varieties. The wheat seeds were inoculated with nitrogen fixing microbes using sugar solution in room condition and were sown during November, 2015. The seeds were obtained from previously treated seeds with total twelve (12) treatments i.e recommended N (120 kg ha^{-1}), *Azotobacter* alone, *Azospirillum* alone, *Azotobacter*+25% N, *Azospirillum* + 25% N, *Azotobacter* +50% N, *Azospirillum*+ 50% N, *Azotobacter* + 75% N, *Azospirillum*+ 75% N, *Azotobacter*+ 100% N, *Azospirillum*+ 100% N and control (no nitrogen and nitrogen fixing microbes). After harvesting the wheat varieties. Seeds were cleaned and were subjected to different vigor and viability tests.

Standard germination test

The experiment was conducted in petri dishes of 35.0 cm in diameter. Seeds were surface sterilized with 0.2% mercuric chloride solution for five minutes and then washed 2 to 3 times with sterilized distilled water. A double layer of blotting paper was laid down in each Petri dish, and thereafter 100 healthy seeds of uniform size were placed at an equal distance on moist blotting paper. Observations were made after every 24 hours and sprayed with distilled water in order to maintain the moisture level. The following parameters were studied in this experiment: Seed leakage: The amount of electrolytes leakages from seeds was made on sample of 100 seeds from each treatment. Each sample of 100 seeds was placed in flask to which 200 ml of distilled water is added. The flask was kept at 20 C^{O} for 24 hours after that electrical conductivity of soaked water is measured by using conductivity meter. Conductivity is expressed in micro-siemens per gram of seed (Ashraf et al., 1999).Water imbibitions of wheat seeds: Imbibitions for each varieties of wheat were studied in petri dishes of 9.0 cm in diameter. Each treatment was replicated three times. Wheat seeds were initially treated with 1.0% mercuric chloride solution for few seconds. A double layer of blotting paper was laid down in each Petri dish, and thereafter 100 healthy treated seeds of uniform size were placed on blotting paper. Before placing, each sample was initially weighed (W1). These Petri dishes were then placed in an Incubator at 30°C. After 24 hours, each sample of seeds were reweighed (W2) one by one. Water absorption was calculated with the following formula (Ting 1980).

$$\text{Water absorption (\%)} = \frac{W2 - W1}{W1} \times 100$$

Standard germination test

Hundred seeds from each various treatments were drawn, placed on petri dish having moist blotting paper, then kept in incubator for germination. The last count were made on tenth day of incubation respectively. Averages of the lots were computed to determine germination percentage (ISTA, 1999), also with daily counting of germinated seeds, determined characteristics such as germination rate and mean germination time (Moshatati and Gharineh 2012). Using following formula for growth rate.

$$\text{Growth rate} = \frac{\text{No of germinated seed} + \ldots\ldots + \text{No of germinated seed}}{\text{Days to first count} + \ldots\ldots + \text{days to final count}}$$

Mean germination time

Mean germination time was calculated on the following equation of Farhadi et al., (2014): MGT= Dn/ n.

Seed vigor index

Seed vigor index is calculated by multiplying germination (%) and seedling length .The seed lot showing the higher seed vigor index is considered to be more vigorous (Farhadi et al., 2014).

Relative water content

Relative water contents were calculated according to the following formula: Where: FW and DW are the fresh weight and the dry weight, respectively (Al-Erwy et al., 2016).

Shoot length (cm)

Hundred seeds each of various treatments sown in the Petri dishes having moist blotting paper and were placed in growth chamber. Shoot length were recorded ten days after sowing. (Shaukat, 2006).

Root length (cm)

Hundred seeds each of various treatments sown in the Petri dishes having moist blotting paper and were placed in growth chamber. Root lengths were recorded 10 days after sowing. (Shaukat, 2006).

Total length of seedling

were also count in each treatment using measuring Tap. Hundred seeds each of various treatments sown in Petri dishes having moist blotting paper and were placed in growth chamber.

Dry weights

were determined after shoot and roots drying in electrical oven at 70°C until constant weight for 24 hour. Average shoot and root dry weights were recorded. (Fallahi et al., 2012).

Root/Shoot ratio dry weight (g)

Collected data from Root and shoot dry weight converted to root/shoot ratio (g) for each treatment.

Statistical analysis

Data were analyzed statistically according to the procedure relevant to RCB design in MS Excel. Least significance difference test was used (Jan et al., 2009).

RESULTS AND DISCUSSION

Seed leakage test

The seed leakage test indirectly evaluates seed vigor, reflecting the integrity of the cell membrane system (Marcos-Filho et al., 1994). Nitrogen fixing microbes and nitrogen ratios significantly affected seed leakage of wheat varieties seeds (Table. 1). Analysis of the data revealed that nitrogen fixers, varieties and their interaction are significantly ($p \leq 0.05$) affected seed leakage. Maximum seed leakage (158.8 dSm^{-1}) was recorded, when *Azotobacter* was applied with 50 % nitrogen as compared to *Azospirillium* with 25 % nitrogen (105.2 dSm^{-1}). The leachates of the seeds that obtained from the crop fertilized with phosphate bio-fertilizer (Ebarvar 3) had the minimum electrical conductivity compare to control treatment (Raissi et al., 2012).

Table 1. Seed leakage (dSm^{-1}), water imbibitions (%), germination (%), growth rate, seed vigor index (SVI), and mean germination time (MGT) of harvested seeds of wheat as affected by nitrogen fixing microbes with nitrogen ratios.

Nitrogen fixing microbes with Nitrogen ratios	Seed leakage	Water imbibitions	Germination percentage	Growth rate	SVI	MGT	RWC
Control	138.8bcd	1.7d	78.5efg	8.2ef	247.1efg	5.5ef	143.1
Recommended N	129.5cde	2.0abcd	84.7bc	10.8bc	274.4bc	5.9bc	259.9
Azotobacter(Alone)	122.2e	2.2ab	81.5cde	10.0cd	266.1cde	5.7cde	250.3
Azospirillum(alone)	123.8de	2.1ab	82.7bcd	10.4bc	276.1bcd	5.8bcd	247.2
Azotobacter+ N 25%	151.2ab	2.0abcd	83.8bcd	10.6bc	297.8bcd	5.9bcd	292.2
Azospirillum +N 25 %	105.2f	2.1abc	94.0a	11.3b	340.7a	6.6a	275.0
Azotobacter+ N 50%	158.8a	2.1abc	95.7a	13.0a	321.0a	6.6a	243.8
Azospirillum +N 50 %	144.3abc	2.3a	85.0b	12.4a	275.8b	6.0b	193.7
Azotobacter +N 75 %	154.2ab	1.8cd	82.8bcd	10.3bc	262.4bcd	5.8bcd	267.7
Azospirillum + N 75 %	154.8a	1.9bcd	80.7def	9.2de	256.0def	5.6de	187.1
Azototbacter + N 100 %	123.8de	2.0bcd	77.3efg	8.4ef	234.1fg	5.4f	196.2
Azospirillum +N 100%	128.5de	1.9bcd	75.8g	8.0f	241g	5.3f	186.0
Lsd	3.3	0.30	3.34	1.05	27.08	0.23	84.7

Table. 2. Shoot length (cm), root length (cm), shoot fresh weight (g), shoot dry weight (g), root fresh wt (g), root dry wt (g), of harvested seeds of wheat affected by nitrogen fixing microbes with nitrogen ratios.

Nitrogen fixing microbes with Nitrogen ratios	Shoot length	Root length	Seedling length	Shoot dry weight	Root dry weight	Seedling dry weight	Root/shoot ratio
Control	2.8b	3.2c	5.9	0.50	0.020	0.52	0.52
Recommended N	2.9a	3.2c	6.1	0.55	0.021	0.57	0.57
Azotobacter(Alone)	3.0cd	3.2c	6.1	0.60	0.025	0.63	0.63
Azospirillum(alone)	3.0bcd	3.3bc	6.3	0.60	0.025	0.63	0.63
Azotobacter+ N 25%	3.4a	3.5ab	6.9	0.66	0.030	0.69	0.69
Azospirillum +N 25 %	3.3ab	3.6a	6.9	0.64	0.030	0.67	0.67
Azotobacter+ N 50%	3.1abc	3.4abc	6.5	0.56	0.025	0.58	0.58
Azospirillum +N 50 %	3.1abc	3.2c	6.3	0.61	0.026	0.64	0.64
Azotobacter +N 75 %	2.8d	3.1c	5.9	0.65	0.031	0.68	0.68
Azospirillum + N 75 %	2.9cd	3.3bc	6.2	0.59	0.030	0.62	0.62
Azototbacter + N 100 %	2.9cd	3.1c	5.9	0.60	0.025	0.63	0.63
Azospirillum + N 100%	2.9cd	3.1c	6.1	0.60	0.025	0.62	0.62
Lsd	0.26	Ns	0.46	Ns	Ns	Ns	Ns

Table. 3. Electrical conductivity (dSm^{-1}), water imbibitions (%), germination (%), growth rate, seed vigor index, mean germination time, shoot length, root length, shoot fresh weight (g), shoot dry weight (g), root fresh wt (g), root dry wt (g), of harvested seeds of Pirsabak-2013 and Shahkar-2013 wheat varieties as affected by nitrogen fixing microbes with nitrogen ratios.

Parameters	Means		Means
	Pirsabak-2013	Shahkar-2013	
Seed leakage (dSm^{-1})	120.9 b	151.6 a	136.25
Water imbibitions	1.9	2.1	2
Germination (%)	86.2a	80.9b	83.55
Growth rate	10.7a	9.8b	10.25
Seed vigor index	289.5a	259.3b	274.4
Mean germination time	6.0a	5.7b	5.85
Shoot length	3.0a	2.9b	2.95
Root length	3.3a	3.2b	3.25
Seedling length	6.3	6.2	6.3
Shoot dry weight	0.59	0.60	0.59
Root dry weight	0.027	0.025	0.026
Seedling dry weight	0.61	0.63	062
Relative water content	221.1b	236.0a	228.5
Root/shoot ratio	0.61	0.63	0.62

Shahkar-2013 demonstrated higher values (151.6 dSm^{-1}) of seed leakage as compared with pirsabak-2013 (120.9 dSm^{-1}) (Table. 3). Lowest seed leakage were observed in Pirsabak-201 (99.3 dSm^{-1}) when seeds treated with *Azospirillum* with 25% nitrogen microbes with nitrogen ratios. Inoculation with Rhizobium and Pseudomonas in Zea mays have been reported to lower the electrolyte leakage (Bano and Fatima 2009). Meditation of the data showed that nitrogen fixing microbes with nitrogen ratios significantly ($p \le 0.05$) affected water imbibitions (Table. 1). Seeds imbibed more water (2.3%), when wheat seeds were treated for *Azospirillum* +N 50 % as compared control (1.7 %) and alone nitrogen applied plots (2 %). No variations were occurred in varieties. However, rapid water imbibition (2.1 %) was recorded for Shahkar-2013 variety and slow water imbibition (1.9%) was observed for variety Pirsabak-2013 (Table. 3). Nitrogen fixing microbes with nitrogen ratios x varieties showed no significant affect on water imbibition. Maximum water were imbibed from seeds of Pirsabak-2013 variety, when seeds were treated with *Azospirillum*+ N 50% as compared to same variety (1.7%) treated with *Azotobacter* + N 75%. Generally nitrogen fixing microbes with nitrogen ratio increased water imbibition (89.1%) as compared to control. While recommended nitrogen alone and nitrogen fixing microbes alone have lowest water imbibitions than collectively nitrogen fixing microbes plus nitrogen ratios.

Germination (%)

Perusal of the data revealed that nitrogen fixing microbes with nitrogen ratios and varieties were significantly ($p \le 0.05$) affected germination (%). Maximum germination (95.7 %) was recorded for wheat seed treated with *Azotobacter*+ N 50 % where as minimum germination was obtained for wheat seed treated with *Azospirillum* + N 100% (Table. 1). Nitrogen fixing microbes with nitrogen ratios x varieties show no significant effect on germination. As Pirsabak-2013 recorded maximum germination (96.7 %) when wheat seeds were treated with *Azospirillum* plus 25 % nitrogen, while Shakar-2013 have lowest (71.0 %) germination when nitrogen used 100 % along with *Azospirillum* (Table. 3). Data showed also that Bio fertilizers enhanced seed germination either under normal or salt stress conditions (Al-Erwy et al., 2016). Highest seed germination (34.2 and 48.9%) were observed in treatment FYM + PSB+ *Azotobacter*+ PGPR in 2007 and 2008 respectively (Pathak et al., 2013). Seed germination on 4th day were highest in T8 (Soil + all microbes) as compared to alone (Jelin et al., 2011). Germination was observed higher in T 3 (Soil + *Azotobacter*) treatment in contrast to other treatments (Mahato et al., 2009).

Growth rate

Mean value of the data showed that nitrogen fixers and varieties significantly affected growth rate of wheat seeds. Highest growth rate (10.7) occurred for variety pirsabak-2013 while lowest growth rate (9.8) was recorded for variety Shahkar-2013 (Table. 3). Highest growth rate (13) was recorded for wheat seed treated with *Azotobacter*+ N 50% whereas lowest growth rate (8) was recorded for wheat seed treated with *Azospirillum* + N 100% (Table. 1). Both microbes enhances growth rate when nitrogen ratio increased but further increasing nitrogen from 50 % showed decreased in growth rate gradually. Same findings were obtained by Dakhly et al., (1997) on other vegetable crops and Sharma et al., (1999) on cabbage, for *Azotobacter* El-Kalla et al*.,* (1997) on faba bean, Hewedy (1999) on tomato and Abo-sedera et al., (2005) on beans for combined treatments (Microbes and chemical fertilizers). Bio-nitrogen fixers x varieties show no significant effect on growth rate of wheat seed. In the highest Nitrogen (*Azospirillum, Azotobacter* and *Pseudomonas)* concentration growth rate increased by 43%, compared in comparison with control. Also, the lowest growth rate occurred in seeds treated with distilled water (Khoshvaghti, et al., 2013). Application of highest Nitragin (*Azospirillum, Azotobacter* and *Pseudomonas)* concentration for corn crop, increased growth rate by 43%, as compared with control (Firuzsalari et al., 2012).

Mean germination time

Arithmetical analysis of the data indicated that nitrogen fixers and varieties were significantly ($p \leq 0.05$) affected mean germination time. Data showed that maximum mean germination time (6.0) was recorded for variety Pirsabak-2013, while minimum value for seed germination time (5.7) was recorded for variety Shahkar 2013 (Table. 1). More mean germination time (6.6) was recorded for wheat seed treated with *Azospirillum* with 50% nitrogen applied, whereas less mean germination time (5.3) was obtained for wheat seed treated with *Azospirillum* with 100% nitrogen applied. Nitrogen fixers with nitrogen ratios x varieties show no significant effect on mean germination time. Mean germination time was increased when increasing nitrogen from 25 to 50 % with nitrogen fixing microbes. After increasing nitrogen from 75 to 100% with both microbes, mean germination time were decreasing. Kennedy and Tychan (1997), accelerate seed germination of corn inoculated with *Azotobacter.* Statistical analysis of the data revealed that nitrogen fixers with nitrogen ratios and wheat varieties were significantly ($p \leq 0.05$) affected seed vigor index. Mean value shown that vigorous seed (7438.3) was recorded for variety Pirsabak-2013 as compared to Shahkar-2013 (6619.2). Maximum seed vigor index (9008.7) was recorded for wheat seed treated with *Azospirillum*+ N 25% where as minimum seed vigor index (5789.3) was obtained for wheat seed treated with *Azospirillum* + N 100%. Nitrogen fixers with N ratios x varieties showed no significant effect on seed vigor index. However, seed vigor index were increased when adding nitrogen from 25 to 50%, while seed vigor index decreasing when increasing nitrogen. The highest enhancement of vigor indexes were obtained from 6CC Nitragin (*Azospirillum*, *Azotobacter* and Pseudomonas).The results of study showed that inoculation of maize seeds with Nitragin (*Azospirillum*, *Azotobacter* and *Pseudomonas*) increased by inoculation (Firuzsalari et al., 2012). Bharathi et al., (2013) concluded that all the seed treatments involving the bio fertilizers recorded high seedling vigor ranging from (904 to 1660) over untreated control.

Relative water content

Nitrogen fixing microbes with different nitrogen ratios significantly affected relative water content. Maximum relative water content (292.2) were recorded in *Azotobacter* along with 25 % nitrogen applied plots. Shaharoona et al., (2006) reported that beneficial soil microbes and organic fertilizers significantly improved growth characters as a result of enhancing plant water content. Beneficial soil microbes enhanced water contents and growth of potato (Awad, 2002); rice (Naseer & Bali, 2007) and wheat (Boutraa et al., 2010). Both varieties and their interaction have no significant effect on relative water content. However, more relative water content (236) were recorded in Pirsabak-2013 variety as compared to Shahkar-2013 (221.1).

Shoot length

Significantly lengthy shoots (3.0cm) was recorded for Pirsabak-2013, while shortest shoots (2.9cm) was recorded for variety Shahkar-2013. Shoot length (3.4cm) was increased for wheat seed treated with *Azotobacter* + N 25% whereas shoot length (2.8cm) were decreased for wheat seed treated with *Azospirillum* + N 100% (Table. 2). Nitrogen fixers with nitrogen ratios x varieties showed no significant effect on shoot length. Lengthy shoots (3.4cm) were recorded when wheat seed were treated with *Azotobacter* + N 25%. Shoot length (35.5 ±0.8), number of leaves (5.6 ±0.6), length (7.8 ±0.8) and width (7.5 ±0.8) of leaves was reported higher in T 3 (soil + *Azotobacter*) treatment followed by T 1 (Soil) and T 2 (Soil + N) (Mahato et al., 2009). Inoculation of corn seeds before planting with 6 cc Nitragin (*Azospirillum*, *Azotobacter* and Pseudomonas) gave rise in shoot length around 54%, compared with un-primed ones (Firuzsalari et al., 2012).

Root length

Root length (3.3cm) was significantly increased for variety pirsabak-2013, while minimum root length (3.2cm) was recorded for variety Shahkar-2013. Root length (3.6cm) were significantly increased when wheat seed treated with *Azospirillum* +N 25 % as compared to lowest root length (3.1cm) was recorded for wheat seed treated with *Azospirillum* + N 100%. Nitrogen fixing microbes with nitrogen ratios x varieties showed no significant effect root length of wheat seeds. However, longest roots (3.7cm) were recorded when *Azospirillum* applied along with 25% nitrogen (Table. 2). Results revealed that in those fennel seeds were inoculated with 4 ml Nitragin (*Azospirillum, Azotobacter* and *Pseudomonas)*, root length increased by 6.5%, compared with the control. Root length density were increased (6.58 inch) when wheat genotype were inoculated with *Azotobacter* and Arbuscular mycorrhizal fungi (Behl et al., 2007). Results revealed that in those corn seeds were inoculated with 6 cc Nitragin (*Azospirillum, Azotobacter* and *Pseudomonas*), root length increased by 35%, compared with the control (Firuzsalari et al., 2012).

Seedling length

Nitrogen fixing bacteria and Nitrogen ratios significantly affected seedling length of wheat seeds. Lengthy seedlings (6.9 cm) were observed in wheat varieties as treated with *Azotobacter* and *Azospirillum* with 25 % nitrogen supplied (Table. 2). While shortest seedlings (5.9cm) were occurred in *Azotobacter* with 75 and 100 % nitrogen applied plots. Wheat varieties showed no significant variation. However lengthy seedlings (6.3 cm) were noted in Pirsabak-2013 variety as compared to Shahkar-2013 (6.2 cm). Nitrogen fixers x varieties have significant on seedling length of wheat varieties. Seedling lengths were enhanced when nitrogen fixing microbes were used along with nitrogen at 25%. Statistically lengthy plants were recorded in Oat plots where *Azotobacter* inoculation was applied and the shortest plant height was recorded in un-inoculated (control) treatment (Saleem et al., 2015). Sorial et al*.,* (1992), have reported that inoculation of tomato with some of the nitrogen fixing bacteria such as *Azotobacter*. Increase in seedling length, stem length and shoot dry weight was crop. In seeds treated with 4 cc Nitragin (*Azospirillum*, *Azotobacter* and Pseudomonas) seedling length increased 5% in comparison to control (179 mm) (Khoshvaghti et al., 2013).

Shoot dry weight

Statistical analysis of the data indicated that shoot dry weight was not significantly affected by nitrogen fixing microbes with nitrogen ratios. Heavier shoots were recorded (0.64g) for *Azotobacter* along with 25% nitrogen treated plots (Table. 2). Whereas lighter shoot weight (0.50g) was observed for control plots. Mandal et al., (2000) reported an increase of 10% - 15% in green and dry forage yield of oat through seed inoculation with *Azotobacter*. Variety Shahkar-2013 produced heaviest shoot weight (0.60g) than that of Pirsabak-2013 (0.59g). *Azotobacter* along with 75 % nitrogen enhanced shoot dry weight (0.76g) in Shahkar-2013 wheat variety (Table. 3). The dry weight of the shoot and root was higher by more than 7% than the control in the case of treatments with bio fertilizer (Bakonyi et al., 2013).

Root dry weight

Perusal of the data indicated that root dry weight was not significantly affected by nitrogen fixing microbes with nitrogen ratios. The effect of varieties on root dry weight was also found non-significant. All possible interactions were non-significant. However highest root dry weight (0.031g) was recorded for *Azospirillum* + N 50% whereas minimum root dry weight (1.4g) was observed for control (Table. 2). No any difference was observed for both varieties (Table. 3). Inoculation of bio fertilizer to rice produced significantly higher root and shoot biomass increased their photosynthetic rate and accumulated higher levels of growth regulating phytohormones (Jelin et al., 2011). Inoculation of Arbuscular mycorrhizal fungi + *Azotobacter* increased root biomass(1.83g) in wheat (Behl et al., 2007).

Seedling dry weight

Seedling dry weight was not significantly affected by Nitrogen fixer microbes, verities and their interaction. However heavier seedling dry weight (0.68g) were observed in *Azospirillum* with 75 % nitrogen applied plots (Table. 2). Lighter seedling dry weights (0.52 g) were observed in control plots. Maximum dry matter % was recorded where *Azotobacter* inoculation was applied to Oat plots and it was followed by *Azospirillum* with % dry matter (Saleem et al., 2015). Shahkar-2013 have heavier seedling dry weight (0.63 g) as compared to Pirsabak-2013 (0.61g) (Table. 3). Tilak et al., (1982) reported positive effects of double inoculation of *Azotobacter* and *Azospirillum* on dry matter of maize and sorghum. Rai and Caur (1998) studied *Azotobacter* and *Azospirillum* and double-inoculation and alone inoculation effects on wheat growth and yield. Inoculation of plants with *Azospirillum* could result in significant changes in various growth parameters, such as increase in plant biomass, nutrient uptake, tissue N content, plant height, leaf size and root length of cereals (Bashan et al*.,* 2004). The results of the experiment concluded that plants inoculated with *Azotobacter* isolates showed better growth response, biomass yield and nutrient content when compared with uninoculated control plants (Sandeep et al., 2011).

Root/shoot ratio

Root/shoot ratio were significantly affected by nitrogen fixing microbes with nitrogen ratios, varieties and their interaction. However, root/shoot ratio were increased (0.69) in those wheat plots which treated with *Azotobacter* with 25 % nitrogen as composed to control plots (0.52) (Table. 2). Root/shoot ratio were highest in Shahkar-2013 (0.63) as compared to Pirsabak-2013 (0.61) (Table. 3). Root/shoot dry weight ratio increased by two percent, due to corn seed primed with 8cc Nitragin (*Azospirillum, Azotobacter* and *Pseudomonas*) (Firuzsalari et al., 2012)

CONCLUSION

It is concluded that nitrogen fixing microbes with nitrogen ratios increased water imbibitions, growth rate, germination, seed vigor index, mean germination time, shoot dry weight, root dry weight, seedling dry weight, shoot length, seedling length, relative water content, root/shoot dry weight ratio and reduced seed leakage as compared to control wheat varieties. *Azospirillum* with 25% nitrogen increased seed vigor index, mean germination time, longest shoots, lengthy roots, seedling length, shoot dry weight, root dry weight and seedling dry weight. Wheat variety Pirsabak-2013 and *Azospirillum* with 25% nitrogen is recommended for highest vigor and viability.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Naseer Gul designed and performed the experiment and also wrote the manuscript. Asim Muhammad performed data analysis, including references and reviewed the manuscript.

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