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ONION SEED PRODUCTION AS INFLUENCED BY FOLIAR APPLICATION OF THIOUREA AND PGPRS AT POST-ANTHESIS STAGE

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ABSTRACT

The onion (*Allium cepa* L.) is a biennial vegetable with regard to seed production. Seed yield is influenced by high temperature at the time of anthesis and seed setting in Punjab. Thiourea (TU) and $PGPR_S$ have been used in different crops to induce stress tolerance and to increase the yield. Two experiments were conducted to study the effect of different concentrations of thiourea (0, 5, 10, 15 and 20mM) and different $PGPR_S$ (Control, PsJN and FD17) on onion seed production. Thiourea and $PGPR_S$ were applied as foliar spray at post-anthesis stage in onion seed crop. Three replications were used under RCBD. In first experiment, maximum values of scape height was 30.367cm in 15mM TU, number of scapes (10.33), thousand seed weight (4.20mg), umbel diameter (5.29mm), weight of seed per umbel (0.34g), seed yield per plant (3.21g), plumule length (7.70cm), radicle length (4.17cm), germination percentage (85.66%), yield of seed per plot (369.85g/ft^{-2}), dry weight (70.70mg) and fresh weight (431.64mg) of seedling in 5mM TU and seed germination duration as 16 days in 15mM of TU. Most of the results were higher in 5mM application of TU. While in second experiment, scape height (29.8cm), umbel diameter (5.29mm) and number of scapes per plant (10.3) were not significantly affected by different treatments yet FD17 showed the best results. Seed yield per plant (3.31g/plant) and yield of seed per plot (296.5g/ft^{2}) were significantly ($P<0.00$) affected by FD17 treatment. Almost all the results of FD17 were superior to PsJN. In conclusion of this research, overall, the performance of thiourea (especially 5mM) was better as compared to others.

Keywords: *Allium cepa*, Thiourea, Seed yield, *Paraburkholderia phytofirmans*

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1. INTRODUCTION

Onion belongs to the plant genus *Allium* and the origin of onion is believed to be Asia (Bagali et al. 2012). Onion is the most important bulbous vegetable crop that has worldwide distribution (Bindu and Podikunju 2015). An estimated 4.3million hectares of land are used to produce 724.25million tons of onions worldwide (Pareek et al. 2017). Onion production has increased in line with the country's population growth (Mahmood et al. 2021; Qazi et al. 2021). The top countries in the world for onion production are China, India, the US, Egypt, Iran, Turkey, and Pakistan. FAO statistics show that Pakistan produced 2.12million tons of onions overall on 0.15m ha of land at an average yield rate of 15.5t ha^{-1}, which is relatively low when compared to potential yields (FAOSTAT 2018). Besides the onion production, Pakistan is also lower in onion seed production. According to a study, Pakistan produces only 2% of the world's onion seed; the remaining 98% is imported from India (Anonymous 2016). The use of inferior seed from a small number of cultivars, inaccurate sowing timing, and uneven fertilization techniques are some of the primary causes of this low productivity in onions. These factors increase production costs and reduce small-land owners' income (Anjum et al. 2011; Haider et al. 2022).

In Pakistan, onion seed production is not very promoted and confined mainly in some areas of Sindh. People don't produce their own seed due to laborious, time consuming and energy demanding task of storing onion bulbs for six months. Many factors affect onion seed production including edaphic and climatic conditions as well as skills in technology. Onion production is reduced by ~ 1.1% during fiscal year 2020-21 (Abbas and Waheed 2021). This crop likes to grow happily in sunny sheltered areas, while cold is pre-requisite for seedling and hot dry bulb ripening growth (Sumner 2019). Production of onion in the country is much higher in rabbi season than the kharif

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season (Government of Pakistan, 2018a; Dwivedi and Asati 2019; Singh et al. 2021). So, the rate of onion produced in rabbi season (April-May) falls due to glut in the market (Government of Pakistan, 2018b). While onion is short in the market during November-December and market price is very high (Fatima et al. 2015). The production areas' rapid increase in temperature is one of the climate's constraints. In turn, temperature of ovaries of flowers showing anthesis, which are isolated in the umbel, may reach up to 40-60°C and leads to abortion of embryos (Tanner and Goltz 1972). As result, seed yield declines drastically and benefit: cost ratio of onion seed producers is also affected. Moreover, this sudden increase in temperature also shorten the duration of seeds setting and grain filling, ultimately decreasing the seed yield and quality in various crops (Tewari and Tripathy 1999).

Thiourea (TU) is a highly efficient regulator that helps crops withstand stress. Crop yield, growth, and seed quality are affected by foliar spraying and treating seeds with various thiourea treatments during the flowering and vegetative stages (Sanaullah et al. 2009; Mehriya et al. 2022). Thiourea is a nitrogen and sulfur containing compound, which has been specifically proven to improve crop growth and productivity (Perveen et al. 2016; Wahid et al. 2017; Zain et al. 2017). Thiourea applied as a spray improves chlorophyll content, partitioning, shoot dry matter, hundred seed weight, and photosynthetic efficiency. Thiourea promote a lighter microbial population in soil and improve the intake of NPK as well as mobilization of important nutrients (Balai and Keshwa 2012). Genes encoding antioxidants, redox state regulation, aquaporins, osmotic adjustment, metabolite biosynthesis, calcium signaling, reactive oxygen species (ROS)-activated ion channels, catalase, and cytochrome P450 are among those whose expression improves on TU application (Srivastava et al. 2009; Demidchik et al. 2010; Srivastava et al. 2011; Patade et al. 2012; Semida et al. 2021). By synchronizing microRNAs and hormones, the application of TU also modifies post-transcriptional regulation to increase the expression of genes related to defense (Srivastava et al. 2017). By reducing oxidative damage and limiting membrane permeability, TU also mitigates the negative effects of abiotic factors, such as high temperatures, droughts, and salinity stress (Srivastava et al. 2010; Kaya et al. 2015; Nouman and Aziz 2022). A biological substance called thiourea keeps the photoassimilates translocation continuing and improves the source-sink relationship (Srivastava et al. 2011; Singh and Singh 2017).

If TU is used as a seed pretreatment, it increases the seed germination; while application as the leaf spray improves the properties of the gaseous exchange, and when used as a means of absorption, root growth and proliferation have increased (Pandey et al. 2012). It shows TU is more effective in the tissues where it is used (Wahid et al. 2017).

PGPRs improve plant growth through various (direct and indirect) methods such as nitrogen fixation, phytohormone production, and soil mineral solubilization (e.g., P, K, Zn and Fe). Plant growth-promoting resistance regulators protect plants from biotic stresses through the production of antibiotics, lytic enzymes, siderophores, and volatile organic compounds. (Vejan et al. 2016).

Different abiotic stresses, such as drought on maize and wheat and high temperatures (32°C) on tomatoes (Naveed et al. 2014a). *Paraburkholderia phytofirmans* strains (PsJN) improve growth of the plants. In lab and greenhouse settings, it increases plant growth and vitality in numerous host plants (Compant et al. 2008).

The fresh and dry weight of plants are positively increased by FD17 treatment. Furthermore, when compared to the uninoculated control maize plants, FD17 increases plant biomass by up to 39% and the number of leaves (vegetative growth) by up to 14%. It also plays a role in plant growth and development and is causing plants to become resistant to fungal attack. Additionally, it has been shown to increase seed yields (Naveed et al. 2014a). Therefore, the current study was conducted to estimate the best dose of thiourea and to evaluate the effect of PGPRs on onion seed production.

2. MATERIALS AND METHODS

2.1. Experimental Site and Conditions

This experiment was executed at Vegetable Research Area, Institute of Horticultural Sciences and Horticulture Seed Lab, Seed Science and Technology Department, University of Agriculture, Faisalabad, Pakistan, during 2016-2017.

2.2. Plant Material and Experimentation

Seeds of onion cultivar "Phulkara" were sown in the Vegetable Research Area during October 2016. Onion bulbs were planted by keeping 1ft bulb to bulb distance on ridges spaced 2.5ft apart. Recommended doses of N, P and K were applied. Crop was sprayed with different concentrations of thiourea i.e., T0 (Control), T1 (5mM), T2 (10mM), T3 (15mM), T4 (20mM) and PGPRs (Control, PsJN and FD17) when 50% umbels had completed anthesis. Onion seed crop was harvested during April 2017.

2.3. Data Collection

2.3.1. Morphological Parameters: Standard methods were used to collect data for radical length (cm), plumule length (cm), scape height (cm), number of scape per plant, weight of seed per umbel (g), umbel diameter (mm), seedlings fresh and dry weight (mg), seed germination percentage, mean germination time (days), yield of seed per plant (g), yield of seed per plot (g ft^{-2}), 1000 seed weight (mg) and seedling vigour index.

Number of scapes per plant was done by counting the scapes per plant. Radical length, plumule length, Height of scape and umbel diameter were measured by Vernier caliper. The height of all umbels was measured and then take average. Weight of seed per umbel, seed yield per plant, seed yield per plot and 1000 seed weight were recorded by measuring balance.

2.3.2 Seed Quality Parameters: Seed germination (%) was done by simply counting of seedlings in petri dishes. Each petri dish contains 25 seeds from each treatment. Mean germination time (days) was recorded by counting the germinated seeds with respect to the time period. Ten seedlings were collected from each treatment to measure the radical and plumule length. Fresh and dry weight of seedlings (mg) was measured by weighing 10 seedlings on electrical balance. Ten seedlings were dried and their weight was recorded. Vigour index of seedling was calculated by following formula:

$$V.I. = Germination\ (\%) \times Seedling\ length\ (cm)$$

2.4. Experimental Layout and Statistical Analysis

Three replications were used under Randomized Complete Block Design (RCBD). Data was analyzed statistically by using the variance technique. At 5% probability Tukey's test was used to compare the treatment means (Steel et al. 1997).

3. RESULTS

3.1. Experiment 1: Effect of Foliar Application of Thiourea on Onion Seed Production

3.1.1 Effect of Foliar Application of Thiourea on Vegetative Traits: Thiourea treatments significantly improved the vegetative traits including umbel diameter and number of scapes per plant (Fig. 1). Results showed that umbel diameter (5.2mm) and number of scapes per plant (13.3) were maximized by 5mM TU treatment. While scape height (30.3cm) was not significantly affected by TU treatments.

3.1.2. Effect of Foliar Application of Thiourea on Yield Related Traits: Seed yield traits of onion showed somehow non-significant differences for various thiourea treatments as shown in Fig. 1. A 1000 seed weight, seed weight per umbel and seed yield per plant showed non-significant differences under thiourea treatments, although it was highly significant for seed yield per plot in control. 5mM TU (T1) treatment produced higher 1000 seed weight (4.1 mg), yield of seed per plant (3.21g/plant) and seed weight per umbel (2.8g) as compared to other treatments but similar statistically while minimum 1000 seed weight (3.7mg) was observed in control. While seed yield per plant (2.8g) and weight of seed per umbel (0.26g) were observed in (T4) 20mM thiourea treatment. Although seed yield per plot (369.8g/ft^2) was maximum at 0mM Control treatment, while minimum seed yield per plot (304.3g/ft^2) was recorded in 20mM (T4) although it was highly significant.

3.1.3. Effect of Foliar Application of Thiourea on Seed Quality Traits: Seed quality traits of onion were significantly affected by different treatments yet 5mM TU showed the best results as compared to other treatments (Table 1). Maximum vigour index (10.39, 10.37) was observed in 5mM and 10 mM thiourea treatments respectively, while minimum (10.21) was in control. Besides this, radical length (4.1cm), seedlings fresh weight (47.20mg) and seedlings dry weight (7.07mg) were maximized by 5mM TU treatment. Although plumule length, seed germination percentage and mean germination time were observed non-significant for TU treatments.

Table 1: Effect of foliar application of thiourea on onion

Treatments	Plumule Length (cm)	Radical Length (cm)	Mean germination time (Days)	Seedlings Dry weight (g)	Seedling Fresh weights (g)	Seed Germination (%)	Seedling Vigour Index	Scape Height (cm)
Thiourea 0mM	7.61±0.06a	3.9±0.24b	15.0±1.16a	6.35±0.02b	43.16±0.01d	85.3±0.12a	10.21±0.02b	27.6±0.11a
Thiourea 5mM	7.70±0.12a	4.1±0.12a	13.6±0.58a	7.07±0.02a	47.20±0.01a	85.6±0.15a	10.39±0.02a	30.1±0.05a
Thiourea 10mM	7.47±0.16a	3.6±0.12ab	15.0±1.16a	6.86±0.02a	46.95±0.02ab	81.0±1.16a	10.37±0.01a	27.7±0.11a
Thiourea 15mM	7.48±0.12a	3.9±0.26b	16.0±1.16a	6.84±0.02a	45.12±0.01bc	81.0±1.16a	10.32±0.02ab	30.3±0.15a
Thiourea 20mM	7.51±0.13a	3.7±0.12ab	153±0.89a	6.37±0.01b	43.64±0.01cd	81.6±0.12a	10.33±0.02ab	28.0±0.01a

Treatments (Mean±SE) sharing same alphabets are statistically non-significant (P>0.05). Biological replicates (n=3).

3.2. Experiment 2: Effect of Foliar Application of PGPRs on Onion Seed Production

3.2.1. Effect of PGPRs on Vegetative Traits of Onion: PGPRs showed non-significant differences for scape height, umbel diameter and number of escapes per plant. Although, scape height (29.8cm), umbel diameter

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(5.29mm) and number of scape per plant (10.3) were not significantly affected by different treatments yet (T2) FD17 showed the best results. Minimum scape height (26.8cm), and umbel diameter (4.96mm) were observed in control treatment, while number of scape per plant (9.0) was minimum in (T1) PsJN treatment.

3.2.2. Effect of PGPRs on Yield Traits of Onion: Significant differences in yield of seed per plant and yield of seed per plot were recorded among the treatments. Results revealed that yield of seed per plant (3.31g/plant) and yield of seed per plot (296.5g/ft^2) were higher in the FD17 (T2) treatment. While (T1) PsJN showed minimum seed yield per plant (282.1g) and minimum seed yield per plot was noted in (T0) control treatment. But seed weight per umbel and 1000 seed weight were observed non-significant for PGPRs treatments as shown in Fig. 2.

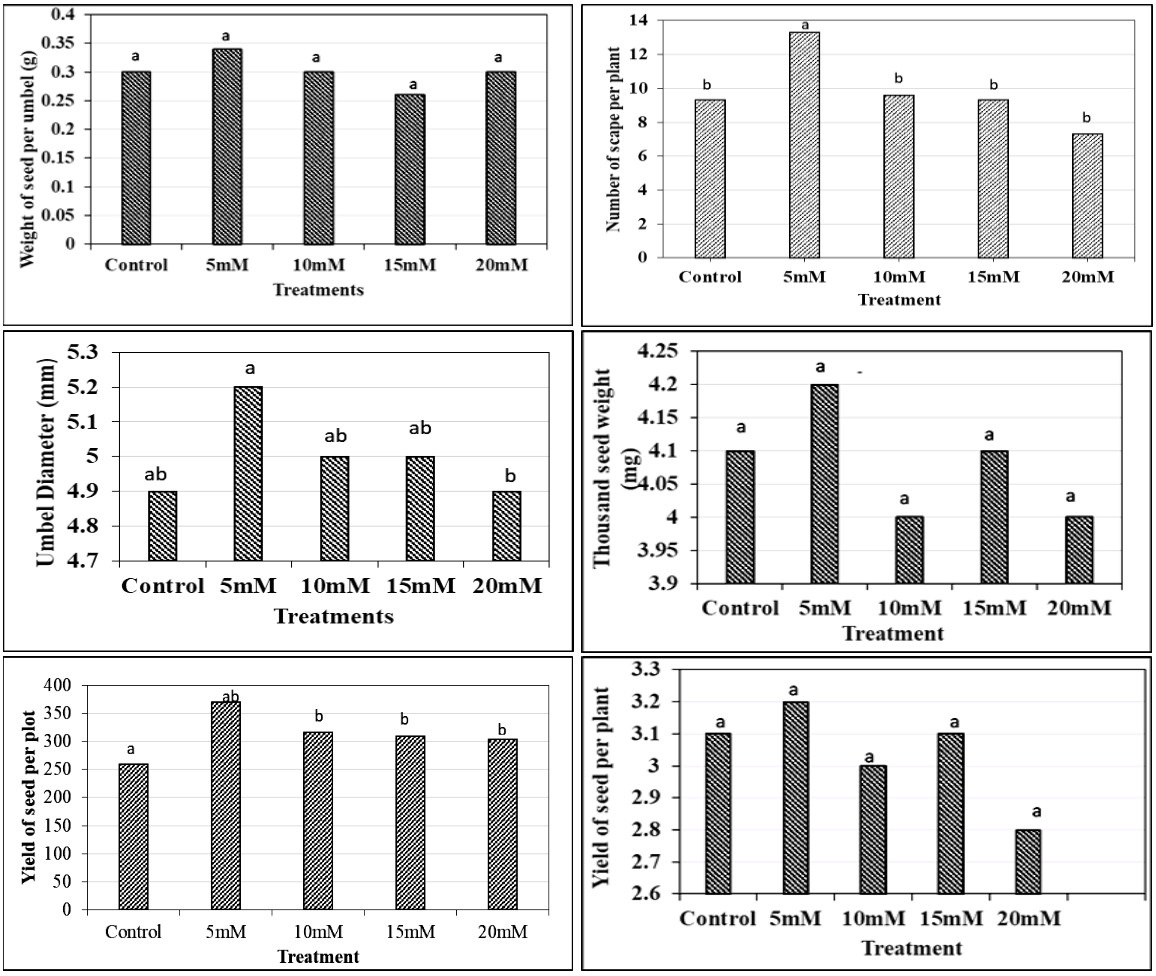

Fig. 1: Effect of foliar application of thiourea on number of scapes per plant, umbel diameter (mm), number of seeds per umbel, 1000 seed weight (mg), yield of seed per plot (g) and yield of seed per plant (g).

Table 2: Effect of foliar application of PGPRs on onion seed quality

Treatments	Plumule Length (cm)	Radical Length (cm)	Mean germination time (Days)	Seedlings Dry weight (g)	Seedlings Fresh weight (g)	Seed Germination percentage	Seedling Vigour Index	Scape Height (cm)
(T0) Control	7.61±0.01a	4.10±0.26b	13±1.15a	6.35±0.02b	43.16±0.01b	85.33±0.01a	999.21±0.00a	26.8±0.06a
(T1) PsJN	7.62±0.00a	4.15±0.02ab	14±1.16a	7.07±0.01a	46.95±0.02a	81.33±0.00a	957.25±0.01a	28.1±0.12a
(T2) FD17	7.70±0.01a	4.16±0.01a	15±0.02a	7.10±0.02a	47.49±0.01a	85.33±0.01a	1012.01±0.02a	29.80.09a

Treatments (Mean±SE) sharing same alphabets are statistically non-significant (P>0.05). Biological replicates (n=3).

3.2.3. Effect of PGPRs on Seed Quality Traits of Onion: Results showed that plumule length, mean germination time, seed germination percentage and seedling vigour index were not significantly affected by different treatments of PGPRs (Table 2). Radical length (4.16cm), seedlings fresh weight (47.49mg) and dry weight (7.19 mg) were maximized by (T2) FD17 treatment. However, radical length (4.10cm), seedlings fresh weight (43.16mg) and dry weight (6.35 mg) were minimum in the (T0) control treatment.

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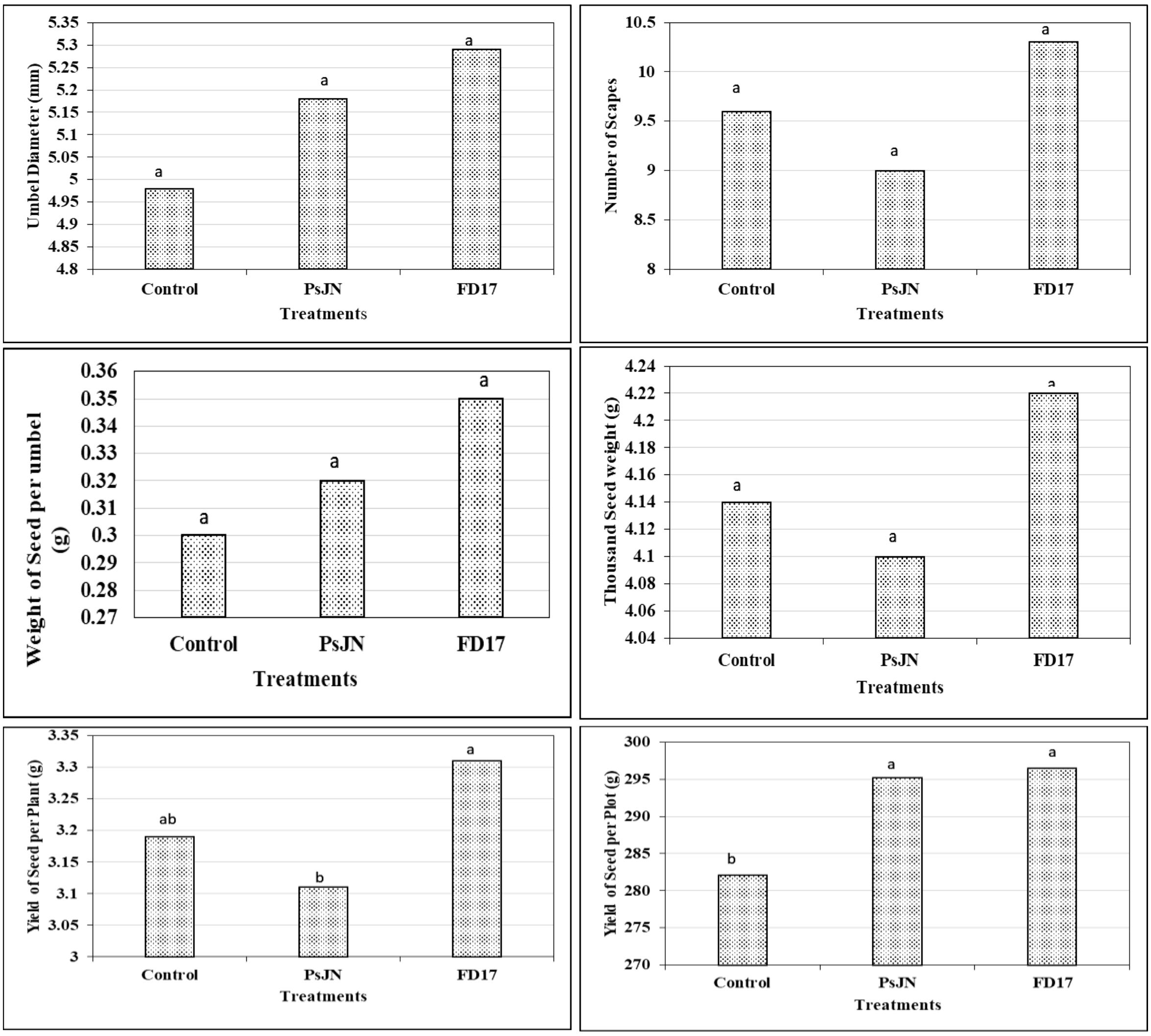

Fig. 2: Effect of PGPRs on umbel diameter, height of scape, yield of seed per plot, yield of seed per plant, number of scapes per plant, weight of seed per umbel.

4. DISCUSSION

In this study, foliar application of thiourea and PGPRs were applied on onion crop when 50% umbel had started flowering. Results showed maximum number of scapes per plant under TU treatment because TU improves the total chlorophyll and net photosynthesis and starch content in the leaves (Singh and Singh 2017). The highest number of scape per plant were also reported by Uddin et al. (2019) in *Allium tuberosum* under the TU treatment as compared to control that corresponds to our findings. Thiourea is a biological compound that increases the source-sink relationship and maintains the photoassimilates translocation (Pandey et al. 2012). Thiourea treatments significantly improved the vegetative traits including radical length, umbel diameter, number of scapes per plant, fresh and dry weight of seedlings. Results showed that radical length (4.1cm), seedlings fresh weight (47.20mg), seedlings dry weight (7.07mg), umbel diameter (5.2mm), number of scapes per plant (13.3), were maximized by 5mM TU treatment. Kaya et al. (2015) also reported that increase in plant biomass due to thiourea application partially confirms our results because a healthy plant can produce healthy seeds which will give rise to healthy seedlings as observed in our experiment. Strain FD17 application which support our results. In the present study, PGPRs showed significant differences for radical length, fresh and dry weight of seedlings. Results showed that radical length (4.16cm), seedling fresh weight (47.49mg) and dry weight (7.19mg) were maximized by (T2) FD17. Naveed et al. (2014a) reported that inoculating with strain FD17 increases plant biomass by 39% and the number of leaves (vegetative growth) by 14%. FD17 increased the maize grain yield up to 42% as compared to the un-inoculated-control maize plants. Of all treatments, seed yield per plant and seed yield per plot were significantly higher in FD17 (3.31g and 296.5g/ft^2) while (T1) PsJN showed minimum seed yield per plant and (T0) showed minimum (282.1g) in control. Weight of seed per umbel and thousand seed weight were not significantly different, although both are maximum in (T2) FD17, Minimum weight of seed per umbel (0.35g) was observed in (T1) PsJN and thousand seed weight (0.30g) in control as shown in Fig. 2. Our results are in line with the findings of Naveed et al. (2014b) who also reported an increase in cob weight of maize due to FD17 application.

5. CONCLUSION

From the present study, it is concluded that thiourea treatments has positive impact on the vegetative traits and seed quality of onion i.e., radical length, umbel diameter, number of scapes per plant, seedlings fresh and dry weight and seedling vigor index. While PGPRs showed positive effect on seed yield and seed quality of onion. From this study, it is recommended to use Thiourea at 5mM concentration and PGPR FD17 on onion seed crop for the improvement of onion seed yield and quality.

Author's Contribution

Conceptualization, K.Z.; methodology, I.M, Y.M. H.M. and S.R.; software, F.S.; validation, M.A.G. and N.F.; writing-original draft preparation, K.Z.; writing—review and editing, K.Z, I.A and F.S.; supervision, K.Z.; project administration and funding acquisition, K.Z. and M.A.G.

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