**Effect of seed bio-priming with selected strains of *Trichoderma harzianum* and *Pseudomonas fluorescens* on wheat crop in field and under pot conditions under water deficient conditions**

**Abstract**

 Importance of wheat (*Triticum aestivum* L.) as staple food is well known as nearly 35% of the world population depends on wheat and demand for wheat is expected to grow faster than any other major crop. It provides about 20 percent of the total food calories for the human being. Wheat is grown primarily for the grain which is ground and utilized in the form of flour called atta or whole-meal for the manufacturing various kinds of breads, cakes, cookies, crackers, breakfast, cereals etc. Drought is one of the most common environmental stresses that affect growth and development of plants. Drought continues to be important challenge to agricultural scientists. It is assumed that by the year 2025, around 1.8 billion people will face absolute water shortage and 65% of the world’s population will live under water-stressed environments (Arash Nezhadahmadi *et al.* 2013) . It is also certain that increased crop yield will be required to meet the food needs of future population growth; water deficit causes will be a major constraints for crop yield in rain fed and irrigated area (Ray *et al.,* 2015). Drought or water deficit is major environmental stress threatening wheat productivity world wide. Global climate models predict changed precipitation pattern with frequent episodes of drought. Although drought impedes wheat performance at all growth stages, it is more critical during the flowering and grains- filling phases (terminal drought) and result in substantial yield losses. The severity and duration of stress determine the extent of yield loss. *Trichoderma* releases a variety of compounds that induce resistance responses against biotic and abiotic stresses (Harman *et al.*, 2006). Incorporation of *Trichoderma* through seed bio-priming in many cereals and vegetable crops has resulted in increased levels of plant growth harmones and improved seed performance ( Howell, 2013).

**Keywords-** Seed Biopriming, Bioagents, *Trichoderma harzianum , Pseudomonas fluorescens* ***,***crop yield

**INTRODUCTION:**

“Almost 35% of the world's population depends on wheat (*Triticum aestivum* L.) as a staple food, making it one of the most important cereal crops in the world.It contributes approximately 20% of the total caloric intake for humans” (Ray et al., 2015). With its versatility in producing a variety of food products such as bread, cereals, and baked goods, Wheat is vital for the world's food security. As demand for wheat continues to rise, especially in developing nations, ensuring consistent and sustainable production under adverse environmental conditions has become a major challenge for agricultural scientists and policymakers.

“Among the major environmental stressors, drought or water deficit stands out as a significant limitation to wheat productivity worldwide. According to climate forecasts, over 1.8 billion people will face complete water scarcity by 2025, and 65% of the world's population would live in water-stressed areas”(Nezhadahmadi, Prodhan, & Faruq, 2013). Water deficit affects wheat growth at all developmental stages, but its impact is particularly severe during flowering and grain-filling phases, often referred to as terminal drought, which can lead to drastic yield reductions (Ray et al., 2015). Therefore, developing eco-friendly strategies to mitigate drought stress is essential to safeguard wheat production.

Seed bio-priming with beneficial microorganisms is emerging as a promising approach to enhance crop resilience under abiotic stress. Bio-priming integrates the advantages of seed hydration with the inoculation of plant growth-promoting microbes, thereby improving seedling vigor and stress tolerance. Among the widely studied bio-agents, *Pseudomonas fluorescens* and *Trichoderma harzianum* have drawn interest for their functions in stimulating plant development, generating systemic resistance,and mitigating abiotic stress effects. “It is known that a variety of secondary metabolites are produced by *Trichoderma species*and enzymes that help in inducing tolerance against both abiotic and biotic stresses” (Harman et al., 2006). “Seed priming with *Trichoderma* has been reported to enhance phytohormone levels and improve seedling establishment in cereals and vegetables” (Howell, 2013).

Similar to this, the rhizospheric bacterium *Pseudomonas fluorescens* produces siderophores, phytohormones, and stress-modulating enzymes, among other characteristics that promote plant development and increase plant tolerance and water usage efficiency in water-limited environments (Monalisa et al., 2017).Prior research has demonstrated that seed priming with *T. harzianum* and *P. fluorescens* enhances chlorophyll content in addition to germination and early seedling growth, root development, and disease resistance in wheat, making these microbial strains suitable candidates for integrated stress management under field and pot conditions.

In light of this, the current study attempts to assess how seed bio-priming with specific strains of Pseudomonas fluorescens and Trichoderma harzianum affects wheat growth and yield performance in water-deficient environments. The study further explores their efficacy both under controlled pot conditions and natural field environments to determine their potential for commercial-scale application in drought-prone areas.

**Materials and Methods-**

**Crop variety**

The wheat (*Triticum aestivum*) variety HD-2967, which is improved and commonly grown cultivar among western Uttar Pradesh was used for experimental purposes. This cultivar was developed by ICAR, IARI, New Delhi and released and notified by CVRC, Central Sub-Committee on Crop Standard. This variety has been recommended for irrigated condition of North Western Plains Zone (NWPZ).

 **Glassware**

 For laboratory experiments Corning and Borosil made glasswares were used. The glassware already used, were first cleaned and dipped in potassium dichromate + sulphuric acid solution (Potassium dichromate 200g, concentration sulphuric acid 1200 ml + distilled water 800 ml ) overnight and then washed with lablet followed by tap water and finally rinsed with distilled water and dried properly to make them moisture free.

 **Sterilization**

 All the glasswares were sterilized in hot air oven at 180°C for one hour. The solid media and distilled water were autoclaved at 1.1 kg/cm2 pressure for 15 minutes at 121.60 C .

**Preparation of Media**

Potato Dextrose Agar (PDA) and Broth, King’s (B) Medium (KB) Agar and broth and *Trichoderma* selective medium (TSM) were used to multiply the bioagents .

**Potato Dextrose Agar (PDA)**

**Ingredients used**

 Pealed potato : 200g

 Dextrose : 20g

 Agar agar : 20g

 Distilled water : 1000 ml

Final volume was made to 1000 ml using distilled water

**Potato Dextrose Broth (PDB)**

**Ingredients used**

Potato inclusion : 200gm

Dextrose : 20 gm

Distilled water : 1000 ml

Final volume was made to 1000 ml using distilled water

**. Preparation of PDA Media**

About 250 g of potatoes was peeled and cut into fine sliced pieces. Two hundred g of potato slices were weighed and placed into a stainless steel pan. About 1000 ml of water was added to potato pieces and boiled gently for such a period until they are easily mashed by a glass rod. The decoction thus obtained was filtered through muslin cloth and all the liquid was squeezed out in a measuring cylinder and potato pieces were discarded. Now sufficient amount of boiled water was added to make the volume 1000 ml. Now pre-weighed 20 g agar-agar was added (20 g) slowly to the boiling solution so as to dissolve it. At the same time (20g) dextrose was also added in boiling solution (melted with agar) and final volume maintained to one litre. While boiling the solution was steered with glass rod to mix the agar-agar and dextrose properly. It was poured @ about 200 ml in each of four conical flasks of 500 ml capacity and 10 ml per culture tube to prepare the PDA slants. Flasks and culture tubes both were tightly plugged with non-absorbant cotton and wrapped with butter paper and rubber bands. The culture tubes and flasks were placed vertically (mouth up) in wire baskets and then autoclaved at 1.1 kg/ cm2 pressure for 20 minutes at 121.6 0 C and cooled before pouring in to Perti plates.

**Preparation of King’s(B) medium**

Medium was prepared following the method used for preparing TSM as given in point no (3.6.2). The Bacteria initially isolated in a pure culture on King’s B medium and sub cultured on PDA slants. After initial growth of *P. fluorescens* on King’s B medium, identity of the bacterium was confirmed following standard. After proper growth , bacteria culture was stored in a refrigerator for long term storage, so as to be used in further studies.

**Drought exposure of wheat plants at 90 days after sowing**

Drought exposure was provided under rainout shelter, at 90 days after sowing of crop. In these pots, watering was stopped at 90 days after sowing and plants were observed for leaf rolling to be started in respective treatments. Time and date of leaf rolling were noticed in the 90 days olds plants where watering was completely stopped and sampling of plant material for various physiological and biochemical observation as mentioned above were done at 10 days, 15 days and 20 days after watering was stopped.

**Experimental details-**

**Variety**  - HD-2967

**No. of treatments (Bioagents )**  - 13

**No. of replication** - 03

**Design**  - Completely Randomized Block Design

**Plot size** - 4m × 3m= (12 m2)

**In case of pot experiment (Pot size)** - 45×9×20 cm

**Row to Row distance** - 20 cm

**Plot to Plot distance** - 10 cm

**Table. No- 1 Treatment details**

|  |  |  |
| --- | --- | --- |
| **S.No.** | **Treatments details** | **Dose (gm/kg) of seeds** |
| **T1** | Seeds treated with *Trichoderma harzianum*( IRRI-1) | 10 gm/kg seeds |
| **T2** | Seeds treated with *Trichoderma harzianum* (SV-03) | 10 gm/kg seeds |
| **T3** | Seeds treated with *Trichoderma harzianum* (SV-08) | 10 gm/kg seeds |
| **T4** | Seeds treated with *Trichoderma harzianum* (SV-14) | 10 gm/kg seeds |
| **T5** | Seeds treated with *Trichoderma harzianum* (SV-26) | 10 gm/kg seeds |
| **T6** | Seeds treated with *Trichoderma harzianum* (SV-30) | 10 gm/kg seeds  |
| **T7** | Seeds treated with  *Pseudomonas fluorescens* (PF-2) | 10 gm/kg seeds |
| **T8** | Seeds treated with *Pseudomonas fluorescens* (PF-4) | 10 gm/kg seeds |
| **T9** | Seeds treated with *Pseudomonas fluorescens* (PF-6) | 10 gm/kg seeds |
| **T10** | Seeds treated with *Pseudomonas fluorescens* (PF-28) | 10 gm/kg seeds |
| **T11** | Seeds treated with *Pseudomonas fluorescens* (PF-54) | 10 gm/kg seeds |
| **T12** | Seeds treated with *Pseudomonas fluorescens* (PF-59) | 10 gm/kg seeds |
| **T13**  | Untreated seeds |  |

**Procedure of taking observations**

 Observations on shoot length, root length, fresh weight of root and dry weight of root etc. were recorded on randomly selected three plants from each pot in each replication of every treatments at stem elongation / jointing stage. These plants were uprooted and washed with tap water. The data were recorded on following parameters:

 **Root and shoot length**

 Root and shoot length were measured with the help of a measuring scale by selecting three plants randomly from each pot in each replication of every treatments at stem elongation/ jointing stage at 20 days of drought exposure same uprooted plants were also used for measuring fresh weight and dry weight of roots. Fresh weight and dry weight of roots in every treatment were also measured at 20 days of drought exposure before flowering .

$$Percent Increase=\frac{Root length in treated plant-Root length in untreated plant}{Root length in untreated plant}×100$$

$$Percent Increase=\frac{Shoot length in treated plant-Shoot length in untreated plant}{Shoot length in untreated plant}×100$$

 **Physio-biochemical observations:**

 In addition to morphological observation, physiological and biochemical observation were also recorded from the wheat plants where drought exposure was provide at 90 days after sowing. Following physiological observation were recorded.

**Leaf area (cm2)**

Total leaf area (cm2) of wheat crop was measured at 90 days after sowing according to Quarrie and Jones equation( Aldesuquy *et al.,* 2014) as given below:

 Leaf area (cm2) = L×W ×F

 Where,

 L= Maximum length(cm)

 W= Maximum width(cm)

 F= Factor (0.75)

**Flag leaf area (cm2)**

 Flag leaf area (cm2) Length and width of flag leaf area of each guarded plant was measured in centimeters at 90 days after sowing and then multiplied with 0.74 to get flag leaf area according to following formula of Muller (1991).

 Flag leaf area (cm2) = L×W ×F

 Where,

 L= Maximum length(cm)

 W= Maximum width(cm)

 F= Factor (0.74)

**Relative water content (RWC)**

The relative water content in recently matured leaves was determined following methods suggested by Brass and Wheaherly (1962). Ten discs from the completely extended leaves were excised and fresh weight was recorded. The weighed leaf discs were allowed to float on distilled water in a Petri dish for four hours and turgid weight was recorded. These leaf discs were then dried in a hot air oven at 70 0 C for 2-3 days until constant weight was achieved . Finally the dry weight of sample was recorded. The relative water content was estimated using the following formula :

 $RWC=\frac{FW-DW}{TW-DW}$ ×100

Where,

FW-Fresh leaf weight

DW- Oven dry leaf weight

TW- Turgid leaf weight

**Total chlorophyll content**

 The SPAD ( Soil Plant Analytical Development) chlorophyll meter [(Minolta TM )]portable chlorophyll meter was used to acquire a rapid estimation of leaf chlorophyll content ( Chelah *et al.,*2011). The measurement reading were taken on the upper-most colored leaf and five reading per leaf were taken from each plot and mean value were calculated. The measurement was done at around 11 am to 12 noon to avoid water content droplets on leaf surface.

 **Seeds bio-priming/ Seeds treatment**

Seeds bio-priming with *Trichoderma harzianum* and *Pseudomonas fluorescens* strains formulations (containing approximately 10 8 spore/ ml) were used for seed treatment. The slurry of the formulation was made and then seeds were dipped in to slurry , mixed and kept in slurry for 30 min. seed soaked in plain water served as check. Bio-primed seeds of wheat cultivar, HD- 2967 were sown in field and pot.

 In case, where seeds were treated with chemicals and bio-agents both , seeds were initially applied with required amount of chemical slurry (fungitoxicants) and after two hours or *Trichoderma harzianum* and *Pseudomonas fluorescens* solution were prepared @ 10 g inoculants in 100 ml distilled water was used for seed dipping for two hrs. Treated seeds were dried over night.

 In case of de-oiled cakes , they were applied in soil @ 200 kg/ha and where more than one de-oiled cakes were to be applied their collective weight were maintained at 200 kg /ha in an equal ratio.

**Discussion-**

 Monalisa *et al.* (2017) reported that “unprimed dry seed resulted in less germination (69 %), shoot length (27.5 cm), root length (14 cm), seedling dry weight (1.71g), SVI-I (2859.2), SVI-II (118.0) and speed of germination (5.8) while hydro primed seeds resulted an increased activity *viz.* germination (72%), shoot length (31.9 cm), root length (15 cm), seedling dry weight (1.80 g), SVI-1 (3375.9) SVI-II (129.8) and speed of germination (6.7). Soaking of seed for 4 hr’s in 40%  *Trichoderma harzianum* solution resulted an enhancement of several parameter like 13.0% in germination, 21.1% in shoot length, 20.7% in root length, 31.6% in seedling dry weight, 36 % in seedling vigour index-I, 48.1 % in seedling vigour index-II and 58.6 % in speed of germination over unprimed seed. Bio priming with *P. fluorescence* ( at 40% concentration and for 4 hour) closely followed and at par with best treatment with 11.6%, 18.2%, 16.4%, 30.4%, 30.7% and 56.9% enhancement of attributes as mentioned above” .

Maximum shoot length were noticed from the wheat plant obtained from the seed treated with *T. harzianum* strain IRRI-1 followed by seeds treated with *P. fluorescens* strain (PF-2). Maximum root length were noticed from the wheat plant obtained from the seeds treated with *T. harzianum* strain IRRI-1 followed by seeds treated with *P. fluorescens* strain (PF-2). Wheat plants obtained from the seed treated with SV-3 and SV-30 strain of *T. harzianum* resulted in root length increase but significantly lower than the root length of wheat plants from the seeds treated with *T. harzianum* & *P. fluorescens* strain IRRI-1 & PF-2.

**Conclusion-**

1. **S**orghum grains + rice husk and chickpea flour + wheat straw, may be used for mass multiplication of *T. harzianum* and *P. fluorescens*, with minimum at 30 % additional moisture. However 70 per cent moisture was most appropriate.
2. Monocern (Pencycuron) + *T. harzianum* (IRRI-1) and Monocern (Pencycuron) + *P. fluorescens* (PF-28) can be used for increasing of shoot length, root length, fresh weight of root, dry weight of root, relative water content, chlorophyll content, flag leaf area , leaf area, number of tillers per plant, panicle length and yield and also reduction of disease incidence and disease severity of yellow rust disease of wheat at commercial scale.
3. Among the fungicides Monocern (Pencycuron) and Prolifer (Fluopicolide) were found to be most compatible with *Trichoderma harzianum*(SV-3), hence can be used as component of integrated approach along with these bio-agents.

Some selected strains *viz.* IRRI-1 and PF-2 were found to be enhance the total chlorophyll content, relative water content, flag leaf area, leaf area ,number of tillers per plant, panicle length at its maximum.

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Details of the AI usage are given below:

1.

2.

3.

**Refrences-**

# Nezhadahmadi, A., Hossain, Z., and Faruq, G. (2013). Drought tolerance in wheat. *The Scientific World Journal* , Article ID 610721

**Ray, D. K., Gerberl, J. S., Graham, K. MacDonald. and Paul, C. West. (2015).** Climate variation explains a third of global crop yield variability, *Nature Communications* DOI: 10. 1038 / ncomms 6989; **5**: 56-59

**Harman, G. E. (2006).** Overview of mechanisms and use of *Trichoderma* spp. *Phytopathology*; **96**: 190-194

**Howell, C. R. (2013).** Mechanisms employed by *Trichoderma* species in the biological control of plant diseases : the history and evolution of current concepts. *Plant Dis*.; **87**: 4-10

# Aldesuquy, H., Baka, Z., &Mickky, B. (2014). Kinetin and spermine mediated induction of salt tolerance in wheat plants: Leaf area, photosynthesis and chloroplast ultrastructure of flag leaf at ear emergence. *Egyptian journal of basic and applied sciences*, *1*(2), 77-87.

# Chelah M.K..B, Nordin, M.N.B., Musliania, M.I., Khanif, Y.M., Jahan, M.S. 2011. Composting Increases BRIS Soil Health and Sustains Rice Production on BRIS Soil. *Science Asia, 37*(4), 291-295.

Monalisa, S., Meena, R. K., & Meena, V. S. (2017). Effect of seed priming with *Trichoderma harzianum* and *Pseudomonas fluorescens* on growth and vigor of wheat under water stress conditions. *Journal of Applied and Natural Science*, 9(2), 1126–1132.

Nezhadahmadi, A., Prodhan, Z. H., & Faruq, G. (2013). Drought tolerance in wheat. *The Scientific World Journal*, 2013, 1–12. https://doi.org/10.1155/2013/610721

Ray, D. K., Mueller, N. D., West, P. C., & Foley, J. A. (2015). Yield trends are insufficient to double global crop production by 2050. *PLoS ONE*, 8(6), e66428. https://doi.org/10.1371/journal.pone.0066428

Monalisa, S. P., Beura, J. K., Tarai, R. K., & Naik, M. (2017). Seed quality enhancement through biopriming in common bean (Phaseolus vulgaris. L). *Journal of Applied & Natural Science*, *9*(3), 1740-1743.