Analysis 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. A prevalent environmental stressor that impacts plant growth and development is drought. Drought is a significant concern for scientists studying agriculture. An estimated 1.8 billion people will experience a complete water deficit by 2025, and 65% of people on Earth are predicted to live in water-stressed settings(Arash Nezhadahmadi*etal.* 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). One of the main environmental stresses endangering wheat productivity worldwide is drought or water scarcity. Changes in precipitation patterns and frequent drought events are predicted by global climate models. Drought hinders wheat growth at every stage, but it is particularly severe during the blooming and grain-filling stages (terminal drought), which causes significant output losses.The amount of yield loss is determined by the stress's duration and intensity.Numerous substances released by Trichoderma cause resistance reactions to both biotic and abiotic stressors(Harman *et al.*, 2006). Trichoderma has been added to various grains and vegetable crops through seed bio-priming, which has enhanced seed performance and raised plant growth hormone levels(Howell, 2013).

**Keywords-** Seed Biopriming, Bioagents

**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. ICAR, IARI, New Delhi, produced this cultivar, and CVRC, the Central Sub-Committee on Crop Standard, released and announced it. 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 previously used glassware was cleaned, dipped in a solution of potassium dichromate and sulfuric acid (200g of potassium dichromate, 1200 ml of sulfuric acid concentration, and 800 ml of distilled water) for an entire night, rinsed with distilled water, and then dried thoroughly to remove any remaining moisture.

 **Sterilization**

Every piece of glassware was sanitized for an hour at 180°C in a hot air oven. For 15 minutes at 121.6o C, the solid medium and distilled water were autoclaved at 1.1 kg/cm2 of pressure.

**Preparation of Media**

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

**(PDA) Potato Dextrose Agar**

**Ingredients used**

 Pealed potato : 200g

 Dextrose : 20g

 Agar agar : 20g

 Distilled water : 1000ml

Using distilled water, the final volume was adjusted to 1000 ml.

**Potato Dextrose Broth (PDB)**

**Ingredients used**

Potato inclusion : 200gm

Dextrose : 20 gm

Distilled water : 1000 ml

Using distilled water, the final volume was adjusted to 1000 ml.

**. (PDA) Potato Dextrose Agar Media Preparation**

Potatoes weighing about 250 g were peeled and thinly sliced.After being weighed, 200 g of potato slices were put in a stainless-steel pan. Potato slices were simmered gently for a while until they could be readily mashed with a glass rod after adding about 1000 milliliters of water.The decoction thus obtained was filtered through muslin fabric and all the liquid was squeezed out in a measuring cylinder and potato bits were discarded. At this point, enough boiling water was added to reach the 1000 ml volume. To dissolve it, 20 g of pre-weighed agar-agar was now gradually added to the boiling solution. In addition, 20g of dextrose was added to the boiling solution (melted with agar) at the same time, keeping the final volume at one liter.To ensure that the agar-agar and dextrose were correctly mixed, a glass rod was used to direct the solution while it was boiling. To produce the PDA slants, it was put at a volume of approximately 200 ml into each of four conical flasks with a 500 ml capacity, with 10 ml each culture tube. Rubber bands and butter paper were used to secure the non-absorbent cotton plugs into the flasks and culture tubes. After being positioned in wire baskets vertically (mouth up), the culture tubes and flasks were autoclaved for 20 minutes at 121.60 C at 1.1 kg/cm2 of pressure. They were then allowed to cool before being transferred to Perti plates.

**King’s(B) mediumPreparation**

The procedure described in point no. (3.6.2) for preparing TSM was followed in the preparation of the medium. The bacteria were subcultured on PDA slants after being first isolated in a pure culture on King's B medium. Following the normal procedure, the identity of *P. fluorescens* was verified following its initial development on King's B medium. Following appropriate growth, the bacterial culture was kept for long-term use in subsequent research in a refrigerator.

**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.

**Procedure of taking observations**

 In each replication of each treatment at the stem elongation/joining stage, three randomly chosen plants from each pot were observed for root length,shoot length, dry weight of the root, fresh weight of the root, , etc. After being uprooted, these plants were cleaned with tap water. The following parameters were used to record the data:

 **Root and shoot length**

 Three plants were chosen at random from each pot in each replication to measure the length of the roots and shoots using a measuring scaleof 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:**

 Ninety days following sowing, the wheat plants that had been exposed to drought were observed morphologically as well as physiologically and biochemically. The physiological observations that followed were noted.

**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)**

 Area of the flag leaf (cm2) Each guarded plant's length and width of flag leaf area were measured in centimeters at 90 days after sowing, and the results were then multiplied by 0.74 using Muller(1991) formula.

 Flag leaf area (cm2) = L×W ×F

 Where,

 L= Maximum length(cm)

 W= Maximum width(cm)

 F= Factor (0.74)

**Relative water content (RWC)**

Using techniques recommended by Brass and Wheaherly(1962), the relative water content of freshly developed leaves was measured. Ten discs from the completely extended leaves were excised and fresh weight was recorded. After four hours of letting the weighed leaf discs float on distilled water in a Petri plate, the turgid weight was noted. After that, these leaf discs were dried for two to three days at 70 degrees Celsius in a hot air oven until their weight remained consistent. Lastly, the sample's dry weight was noted. The following formula was used to estimate the relative water content:

 $RWC=\frac{FW-DW}{TW-DW}$ ×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*etal.* (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%  *Trichodermaharzianum* 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 .

**Conclusion-**

1. **S**orghum grains + rice huskand 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*Trichodermaharzianum*(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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