

## Efficacy of fly-ash based bio-fertilizers vs perfected chemical fertilizers in wheat (*Triticum aestivum*)

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### Abstract

Fly-ash was evaluated for possible use as carrier for *Azotobacter* and *Azospirillum* formulation. *Azotobacter* and *Azospirillum* strains were isolated from healthy wheat rhizosphere soil and bio-formulated in fly-ash (300 meshes). Fly-ash based *Azotobacter* and *Azospirillum* formulation alone and in combination with chemical fertilizer was evaluated for bio-efficacy on wheat. Population of *Azotobacter* and *Azospirillum* was also evaluated in treated soil. The results of the studies showed that, seed treatment with *Azotobacter* and *Azospirillum* and soil treated with chemical fertilizer alone and in combination significantly enhanced the seed germination, plant height, plant biomass and crop yield compared to control. Chemical fertilizer treated wheat plant observed more effective bio-efficacy than bio-fertilizers treated wheat but reduced (destroyed) the microbial population in soil. Whereas *Azotobacter* and *Azospirillum* treated soil observed significantly enhanced the microbial population with slightly lesser plant growth as compared to chemical fertilizer. In the present study it was showed that utilization of fly-ash as carrier in bio-fertilizer formulations emerged as safe and effective alternatives. Use of fly-ash as carrier in these formulations is an effective way of utilization of problematic fly-ash waste in a useful manner.

*Keywords:* *Azotobacter*, *Azospirillum*, bio-efficacy, fly-ash, wheat

### 1. Introduction

Huge amount of fly-ash is being generated from coal based thermal power plants in our country. Many Scientist have carried the study of different aspects of fly-ash and its utilization. Only little of it is being utilized in different ways. Although fly-ash contains traces of toxic elements and heavy metals but due to having some macro and micro nutrients in it and its physical characteristics it can be used as soil amendments and soil conditioner and enhance plant growth (Table – 1A, see appendix). Toxic effect of coal-ash is found to be insignificant and concentration of toxic elements is found to be within permissible limit on utilization in some plantation work (Kumar and Chauhan, 2008).

Although there are many ways to consume the fly-ash generated from thermal power plants but the steps taken in this direction is meager. In the present proposal it is proposed to explore utilization of fly-ash as carrier in bio-fertilizer formulations. With growing concern for degrading environment due to excessive use of chemical fertilizers for nutrient management, bio-fertilizers have emerged as safe and effective alternatives. Their use in Integrated Nutrient Management (INM) programs of the country is being increasingly recommended / promoted. Presently, these formulations are mostly lignite, Dolomite or charcoal powder based. Use of these carrier materials adds to cost of the product without giving any advantage to soil or crops. In fact, long term continuous use of Dolomite etc may even have adverse effect in this reference. Use of fly-ash as carrier in these formulations is expected to be an effective way of utilization of problematic fly-ash waste in a useful manner. Investigations will however have to be conducted, to evaluate the survival / shelf-life, product bio-efficacy etc of bio-fertilizers when formulated in fly-ash as a carrier (Gaiind and Gaur, 2004).

In India, large quantities of fly ash are being generated, as most of our energy demand is met through coal based thermal power stations. The fly ash generation is expected to grow further as coal would continue to remain as major source of energy at least for

next 25 years. The fly ash, which is a resource material, if not managed well, may pose environmental challenges. Fly-ash utilization program (FAUP) has been undertaking various projects/activities for technology development/demonstration, disseminating the information, creating awareness, facilitating multiplier effects, providing inputs for policy interventions etc. in the area of safe management & gainful utilization of fly ash (FAUP, 2007).

Over 160 million ton of fly-ash is produced each year and much of it gets dispersed into the atmosphere before deposition. The fine particles of fly-ash by virtue of their lightness can become air – borne and need to be managed well. It is also a major source of discharge of heavy metals such as cadmium, chromium, lead etc. into the rivers and estuarine sediments, which are in the vicinity of power stations. The thermal discharge into water bodies may lead to adverse effects in the ecosystem, because of close ecological links. Utilization of inferior grade of coal increases coal consumption in the power stations; thus, fly-ash utilization is being also promoted in agriculture. The positive results through various trials conducted by National Fly-ash Utilization program (Kumar et al., 1998).

In the present study, the objective was to see if the method could be used for fly-ash inoculants routinely prepared in large quantities for agricultural use. It was also to see if individual strains from mixed inoculants could be identified. Fly-ash based inoculants were selected as test materials for several reasons:

- Fly-ash is regularly generated as a by-product by any established coal or thermal power stations.
- Fly-ash is one of the most challenging waste materials in the world.
- Fly-ash leads to degradation of arable land and contaminate air, water, soil and organisms in the zone of influence.
- The large quantity of fly-ash produced have created enormous environmental problem.

Wheat is an important cereal crop of India and suitable for cultivation in Rabi season. Nature has designed and provided a circular loop for regular flow of nutrients for sustainability of agriculture. But urbanization and industrialization have broken loop and encouraged a linear flow. Further, with the shrinking cultivable land resources, the demand to produce more and more food per unit area has resulted agriculture becoming heavily dependent on chemical inputs viz. chemical fertilizers - as source for plant nutrients. Increasingly and indiscriminately use of chemical fertilizers may affect soil health and may lead to a negative impact on soil productivity by destroying so many microorganisms which were beneficial to farmers. Hence for sustainable Agriculture, all our efforts should be stream lined to protect and maintain soil health. In this context, now-a-days bio-fertilizers are gaining importance in agriculture (Kumar et al., 2009).

Application of *Azotobacter* and *Azospirillum* inoculants in cultivation of wheat is being recommended as well as commonly practiced for nitrogen source. The present study was undertaken to find out the possibility of using bio-fertilizers either alone or as supplements to chemical fertilizers for wheat. *Azotobacter* and *Azospirillum* are nitrogen fixing bacteria, present in rhizospheric soil. *Azotobacter* produces B-vitamins, Indole acetic acid, gibberellins, cytokinines etc. and increase the activity of beneficial rhizosphere bacteria. *Azospirillum* penetrates and colonizes the roots as well as the tissues of the plant and release some plant growth hormones which promote crop growth.

## 2. Materials and Methods

### *Isolation Azotobacter and Azospirillum strains*

*Azotobacter* and *Azospirillum* strains were isolated from healthy wheat rhizosphere on Ashbey's Agar medium and NFB medium respectively, using "Soil dilution method" (Aneja, 2002).

### *Multiplication and Bio-formulation Development*

*Azotobacter* and *Azospirillum* strains were grown on Ashby's agar and NFB medium respectively for 4 to 5 - days. After checking the culture for purity and proper growth, the culture was transferred in conical flask containing sterilized liquid medium. Broth inoculums were mixed manually in sterilized fly-ash (300 meshes) of 40 % water holding capacity so as to attained final moisture content of formulation to 30 – 35 % (FCO, 2006; Rao, 1986).

### *Microbial population counts*

The population of viable *Azotobacter* and *Azospirillum* were determined at the time of sowing and harvesting using a "Serial dilution pour plate count" methods (Aneja, 2002).

### *Bio-efficacy studies*

Wheat seeds were bio-primed with respective *Azotobacter* and *Azospirillum* formulations (10 g formulation + 10 g farm yard manure + 0.5 g gum Arabic + 50 ml water per 1 kg of seed) whereas chemical fertilizer (Urea) @ 300 kg per ha and sown in small field plots, maintaining 5 replicates of each treatment, with one set of untreated control. Observations were recorded on percentage seed germination after 30 DAS, plant height and plant biomass at 45 DAS and crop yield (Zaidi and Singh, 2004).

## 3. Results and Discussion

### *3.1 Statistical Analysis*

The data obtained were analyzed statistically by using two-way classification Randomized Block Design (Analysis of Variance) for drawing conclusions from the data. The calculated value was compared with tabulated value at 5 % level of probability (Chandel, 2006).

### 3.2 Further results and analysis

The results on bio-efficacy studies included in Table – 1 showed that, seed treatment with *Azotobacter*, *Azospirillum* and soil treated with chemical fertilizer alone or in combination significantly enhanced the seed germination, plant height and plant biomass and crop yield compared to control. Chemical fertilizer treated wheat plant observed more effective bio-efficacy than bio-fertilizers treated wheat but reduced (destroyed) the microbial population in soil. Whereas *Azotobacter* and *Azospirillum* treated wheat observed significantly enhanced the microbial population with slightly lesser plant growth as compared to chemical fertilizer (Table – 2).

Table – 1. Bio-efficacy of various treatments on wheat

| Treatments                                    | % seed germination | Plant height (cm) | Plant Biomass (g) | Yield kg / ha |
|---|--------------------|-------------------|-------------------|---------------|
| T <sub>0</sub> = control                      | 65.40              | 16.88             | 6.50              | 1200          |
| T <sub>1</sub> = 300 kg N / ha as urea        | 85.30              | 20.90             | 10.66             | 4,000         |
| T <sub>2</sub> = <i>Azotobacter</i>           | 84.50              | 19.50             | 11.00             | 3,320         |
| T <sub>3</sub> = <i>Azotobacter</i> + 50 % N  | 87.50              | 21.00             | 11.30             | 3,656         |
| T <sub>4</sub> = <i>Azotobacter</i> + 25 % N  | 84.80              | 19.96             | 9.49              | 3,455         |
| T <sub>5</sub> = <i>Azospirillum</i>          | 82.40              | 17.78             | 9.12              | 3,215         |
| T <sub>6</sub> = <i>Azospirillum</i> + 50 % N | 85.60              | 18.90             | 10.50             | 3,305         |
| T <sub>7</sub> = <i>Azospirillum</i> + 25 % N | 84.00              | 18.20             | 10.00             | 3,125         |
| CD (5%)                                       | 2.07               | 0.69              | 0.68              | 9.25          |

Table–2. Effect of bio-fertilizers and chemical fertilizers on the population of *Azotobacter* and *Azospirillum* at 60 DAS (..... x 10<sup>6</sup> cells / g of soil)

| Treatments                                    | <i>Azotobacter</i>                              |  | <i>Azospirillum</i>                          |  |
|---|---|--|--|--|
|   | Original count (cfu =... x 10 <sup>6</sup> / g) | Transformed count (cfu =... x 10 <sup>6</sup> / g) | Original count (cfu = x 10 <sup>6</sup> / g) | Transformed count (cfu =... x 10 <sup>6</sup> / g) |
| T <sub>0</sub> = control                      | 0.21  | 0.30   | 0.13   | 0.26   |
| T <sub>1</sub> = 300 kg N/ha as urea          | 0.21  | 0.16   | 0.16   | 0.14   |
| T <sub>2</sub> = <i>Azotobacter</i>           | 0.23  | 6.43   | 0.21   | 3.40   |
| T <sub>3</sub> = <i>Azotobacter</i> + 50 % N  | 0.20  | 4.30   | 0.24   | 2.60   |
| T <sub>4</sub> = <i>Azotobacter</i> + 25 % N  | 0.19  | 5.90   | 0.18   | 2.90   |
| T <sub>5</sub> = <i>Azospirillum</i>          | 0.26  | 3.60   | 0.17   | 5.60   |
| T <sub>6</sub> = <i>Azospirillum</i> + 50 % N | 0.22  | 2.10   | 0.19   | 3.20   |
| T <sub>7</sub> = <i>Azospirillum</i> + 25 % N | 0.17  | 2.90   | 0.20   | 4.30   |
| CD (5%)                                       | 1.23  | 0.09   | 1.16   | 0.13   |

The Statistical analysis of data showed that the percentage seed germination, plant height, plant biomass and yield of wheat were significantly increased in all the treatments compared to control. The maximum % seed germination (87.50 %) plant height (21 cm), plant biomass (11.30 g) was observed in T<sub>3</sub> (*Azotobacter* + 50 % N), where as the maximum yeild was measured in T<sub>1</sub> (300 kg N / ha as urea) it might be due to rapid action of Inorganic fertilizer with respect to bio-fertilizers. The population of both *Azospirillum* and *Azotobacter* was comparatively high in all plots irrespective of the treatments. Initial soil analysis also showed the presence of these microbes. This revealed the natural occurrence and further multiplication of both these microbes. The natural occurrence of both *Azospirillum* and *Azotobacter*, initial medium fertility status of the soil, with application of the recommended dose of 300 kg inorganic N ha<sup>-1</sup> contributed to a substantial increase in grain yield of wheat. The results indicate that exclusive

inoculation of bio-fertilizers or inoculation supplemented by partial chemical fertilizer cannot fully match 300 kg inorganic N ha<sup>-1</sup> alone in influencing the productivity of wheat crop. But bio-fertilizers treated soil significantly increased microbial population in soil where as chemical fertilizers treated soil reduced microbial population. The present investigation showed the plots that received recommended dose of 300 kg Inorganic Nitrogen ha<sup>-1</sup> alone produced taller plants thereby higher dry matter. This is due to the ready availability of N during the initial growth stage. The better vegetative growth of plants in plots applied with 300 kg Inorganic N ha<sup>-1</sup> alone resulted in a larger photosynthetic area and thereby more photosynthesis. Further, the efficient translocation of these photosynthates to the reproductive parts resulted in the production of highest grain yield (Table – 1). Similar results on bio-efficacy of *Azotobacter* and *Azospirillum* were reported by (Subbian and Chamy, 1984).

In the present study it was showed that utilization of fly-ash as carrier in bio-fertilizer formulations emerged as safe and effective alternatives. With growing concern for degrading environment due to excessive use of chemical fertilizers for nutrient management. Their use in Integrated Nutrient Management (INM) programs of the country is being increasingly recommended / promoted. Presently, these formulations are mostly lignite, Dolomite or charcoal powder based. Use of these carrier materials adds to cost of the product without giving any advantage to soil or crops. In fact, long term continuous use of Dolomite etc may even have adverse effect in this reference. Use of fly-ash as carrier in these formulations is an effective way of utilization of problematic fly-ash waste in a useful manner.

## APPENDIX

Table – 1A. Physical and Chemical characteristics of fly-ash and soil (FAUP, DST, New Delhi, 2007):

| Properties               | Fly-ash   | Soil      |
|--------------------------|-----------|-----------|
| BD (g cm <sup>-1</sup> ) | <1.0      | 1.33      |
| W.H.C. (%)               | 35-40     | <20       |
| Porosity (%)             | 50-60     | <25       |
| P (%)                    | 0.004-0.8 | 0.005-0.2 |
| K (%)                    | 0.19-3.0  | 0.04-3.0  |
| S (%)                    | 0.1-1.5   | 0.01-0.2  |
| Fe (%)                   | 36-1333   | 10-300    |
| Zn (ppm)                 | 14-1000   | 2-100     |
| Cu (ppm)                 | 1-26      | 0.7-40    |
| Mn (ppm)                 | 100-3000  | 100-4000  |
| B (ppm)                  | 46-618    | 0.1-40    |

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