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A Co – Inoculation Study on the Potential Integrate of Azospirillum and Phosphate Solubilizing Bacteria for Improving Plant Growth and Yield

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Abstract:
Integrated nutrient management strategies involving chemical and biologic fertilizer is a real challenge to stop using the high rates of agrochemicals and to enhance the crop production. The present investigation was aimed at determining the effect of co-inoculation of biofertilizer application on growth and yield of lady's finger (Abel moschus esculentus). Field experiments were carried out for 45 days. Seedling of lady's finger (Abel moschus esculentus) was treated with T0 – control, T1 – Azospirillum, T2 – Phophate solubilizing bacteria, T3 – chemical fertilizer (urea), T4 – Azospirillum + Phosphate solubilizing bacteria and T5 – Azospirillum + Phosphate solubilizing bacteria + Chemical fertilizer (urea). Observations showed that co-inoculation of Azospirillum and Phosphate solubilizing bacteria showed a significant performance in number of leaves, length and breadth of the leaves (leaf area), shoot length, plant height, root length, dry weight and number of pods (yield) when compared with single inoculations and control. The co-inoculation of biofertilizer showed a similar effect with mixed inoculation of Azospirillum, Phosphate solubilizing bacteria and Chemical fertilizer (urea). The pre-cropped and cropped soils were analyzed. The overall result suggests that co-inoculation of Azospirillum and Phosphate solubilizing bacteria improved soil characters, plant growth and yield.

Keywords: *Azospirillum, Phosphate solubilizing bacteria, Chemical fertilizer, urea*

1. Introduction

Plants, like all other living things need food for their growth and development, and they require 16 essential elements. Carbon, hydrogen, and oxygen are derived from the atmosphere, water and soil. The remaining 13 essential elements (nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, zinc, manganese, copper, boron, molybdenum, and chlorine) are supplied either from soil minerals and soil organic matter or by organic or inorganic fertilizers (Al-Khiat, 2006).

Soil contains natural reserves of plant nutrients, but these reserves are largely in forms unavailable to plants, and only a minor portion is released each year through biological activity or chemical processes. This release is too slow to compensate for the removal of nutrients by agricultural production and to meet crop requirements (Jen Hshuan, 2006). In the soil, the mineral nutrients are dissolved in water and absorbed through a plants root. However, the amounts of nutrients in soil are always unpredictable and not enough for plants growth. As a result, primary nutrients NPK which are utilized in the large amounts by crops are commonly found in blended fertilizers nowadays (Ahmad, 2009).

Based on the production process, the fertilizers can be roughly categorized into three types: chemical, organic and biofertilizer. The use of chemical fertilizer or organic fertilizer has its advantages and disadvantages in the context of nutrient supply, crop growth and environmental quality. The advantages need to be integrated in order to make optimum use of each type of fertilizer and achieve balanced nutrient management for crop growth (Jen-Hshuan, 2006).

More recently, a real challenge for the workers in agricultural research field to stop using the high rates of agrochemicals which negatively affect human health and environment. Large quantities of chemical fertilizers are used to replenish soil N, resulting in high costs and severe environmental contamination (Dai *et al*., 2004).

Runoff of synthetic fertilizer can enter the waterways, causing water to be polluted and to lose oxygen. Overtime, chemical fertilizers can degrade the quality of the soil by building up toxins or leaching away natural nutrients, making the soil unfit for growing plants. Using too much fertilizer can damage plants by chemically burning roots and leaves. It is a constant challenge to minimize the use of chemicals in agriculture (Levai *et al*., 2008).

The intensive land use, including the artificial N-fertilizers, in agriculture causes the acidification of soils due to the harvest or leaching of cations. The indirect effect of soil acidity on the presence and availability of toxic ions, such as aluminum, manganese, or other heavy metals, are generally more important to crop production than the direct effect of acidity on the plants. Impacts of soil acidification decrease the number and activity of useful soil organisms, deficiency of magnesium, calcium may occur, phosphorus may become less available. The solubility of several heavy metals may reach toxic level, increasing uptake of heavy metals by crop plants may cause serious health problems of animals and humans (Lévai *et al*, 2008).

Chemical fertilizers pose a health hazard and affect the microbial population in soil by degrading the physical structure of the soil, leading to a lack of oxygen in the plants root zone besides quite expensive and making the cost of production high. In such a situation the role of biofertilizers may be explored as an alternative for enhancing the soil fertility (Chandrasekar *et al*., 2005).

The excessive use of chemical fertilizers has generated several environmental problems, including the greenhouse effect, ozone layer depletion and acidification of water. These problems can be tackled by use of biofertilizers (Saadatnia and Riahi, 2009).

In recent years, biofertilizers have emerged as an important component of the integrated nutrient supply system and hold a great promise to improve crop yield through environmentally better nutrient supplies (Marianna *et al*, 2005). Biofertilizers are cheaper, pollution free, based on renewable energy sources and also improve soil tilth (Saeed *et al*, 2004). The use of biofertilizers, effectively enrich the soil and cost less than chemical fertilizers, which harm the environment and deplete non-renewable energy sources (Contra costa, 2003; Patil, 2010).

Biofertilizers are commonly called microbial inoculants which are capable of mobilizing important nutritional elements in the soil from non-usable to usable form through biological processes **(**Chandrasekar, *et al*, 2005; Selvakumar, 2009). Soil is considered a storehouse of microbial activity, though the space occupied by living microorganisms is estimated to be less than 5% of the total space. Soil microorganisms play an important role in soil processes that determine plant productivity. There is a continuum of bacterial presence in soil, rhizosphere, rhizoplane, and internal plant tissues. Bacteria living in the soil are called free living bacteria as they do not depend on root exudates for their survival. Some bacteria support plant growth indirectly, by improving growth restricting conditions either via production of antagonistic substances or by inducing resistance against plant pathogens (Tilak *et al*, 2005). The interactions among the rhizosphere, the roots of higher plants and the soil borne microorganisms have a significant role in plant growth and development. The organic compounds, released by roots and bacteria, play an important role in the uptake of mineral nutrient (Marianna *et al*, 2005).

For the last one-decade, biofertilizers are used extensively as an eco-friendly approach to minimize the use of chemical fertilizers, improve soil fertility status and for enhancement of crop production by their biological activity in the rhizosphere (Contra costa, 2003; Patil, 2010). Chemical fertilizers are expensive; they disturb the equilibrium of agro-ecosystems and cause pollution to the environment. These problems may be avoided by the use of biofertilizers (Al-Khiat, 2006).

Lady's finger (*Abelmoschus esculentus*) is the only vegetable crop of significance in the Malvaceae family and is very popular in the Indo-Pak subcontinent. In India, it ranks number one in its consumption, but its original home is Ethiopia and Sudan, the north-eastern African countries. It is one of the oldest cultivated crops2 and presently grown in many countries and is widely distributed from Africa to Asia, southern Europe and America. It is a tropical to subtropical crop and is sensitive to frost; low temperature, water logging and drought conditions, and the cultivation from different countries have certain adapted distinguishing characteristics specific to the country to which they belong. (Sathish Kumar *et al.*, 2013).

Hence, the present study aimed at the potential integrate of Azospirillum, phosphate solubilizing bacteria and chemical fertilizer for improving plant growth and yield.

2. Materials and Method

The materials of methods for the study in presented below.

2.1. Biofertilizer

The bacterial strains, *Azospirillum* and *Phosphate solubilising bacteria* were used for the study. The bacterial strains were purchased from the local market in Pavattakkudi village, Thiruvarur district.

2.2. Chemical Fertilizer

The chemical fertilizer, urea was used for the study. The chemical fertilizer was purchased from the local market in Pavattakkudi village, Thiruvarur district.

2.3. Plant

The lady's finger (*Abelmoschus esculentus*) was used for the study. The seeds of ladies-finger (*Abelmoschus esculentus*) were purchased from the local market in Mayiladuthurai town, Nagai district.

- Experimental Period-60 days
- Field Experiment: The field area was selected in Pannainallur village, Thiruvarur district. The field area was measured, length and breadthwise. Before inoculation, the soil was collected, dried and analyzed for the physico-chemical properties. Seed inoculation was done by various alone treatments like *Azospirillum* alone (T1) Phosphate solubilizing bacteria alone (T2), Chemical fertilizer (urea) alone (T3), combined inoculation of *Azospirillum* and Phosphate solubilizing bacteria (T4) and mixed inoculation *Azospirillum*, Phosphate solubilizing bacteria and chemical fertilizer (urea) (T5). Control was also maintained without any fertilizers. The field area was watered daily. After 60 days, the number of leaves, length of the leaves, breadth of the leaves (leaf area), root length, dry weight and number of pods (yield) were calculated. Triplicates are maintained for each treatment.

Label	Treatments
T0	Control (without strains)
T1	*Azosprillum*
T2	Phosphate solubilizing bacteria
T3	Chemical fertilizer (urea)
T4	*Azospirillum* +phosphate solubilizing bacteria (Azo + PSB)
T5	*Azospirillum*+ phosphate solubilizing bacteria+ chemical (urea) fertilizer (Azo + PSB + CF)

Table 1: Experimental Design

3. Results

Table 2: Effect on morphological parameters of lady's finger (*Abelmoschus esculentus*) plants inoculated with bacterial biofertilzer alone, chemical fertilizer alone, combined inoculation of biofertilizers and mixed inoculation of biofertilizer with chemical fertilizer for first 15 days of treatment:

Treatments	Parameters in cm				
	Number of leaves / Plant	Length of the leaves	Breadth of the leaves	Shoot length	Plant height
Control (without fertilizer)	4.5	4	4	7	8
Azospirillum	5	4.9	4.7	8.1	10.0
Phosphate solubilizing bacteria	4.9	4.7	4.4	8	9.3
Chemical fertilizer (urea)	4.7	4.6	4.1	7.8	9
Azospirillum + Phosphate solubilizing bacteria (Azo + PSB)	7.4	6	5	9	10.5
Azospirillum + phosphate solubilizing bacteria+chemical fertilizer(urea)(Azo+PSB+CF)	7.2	5.9	4.9	8.9	10.0

Table 2

Values are expressed in mean ±

This table showed the mean of number of leaves, length and breadth of the leaves (leaf area), shoot length and plant height for the first 15 days of the treatment. The mean of number of leaves, length and breadth of the leaves (leaf area), shoot length, plant height was higher in *Azospirillum* and Phosphate solubilizing bacteria combined inoculation. There was no significant difference in the combined inoculation of *Azospirillum*+Phosphate solubilizing bacteria and mixed inoculation of *Azosprillum* + Phosphate solubilizing bacteria + Chemical fertilizer. A low effects were observed in treatments of *Azospirillium* alone, phosphate solubilizing bacteria alone, chemical fertilizer (urea) alone and control.

Table 3: Effect on morphological parameters of lady's finger (*Abelmoschus esculentus*) plants inoculated with bacterial biofertilzer alone, chemical fertilizer alone, combined inoculation of biofertilizers and mixed inoculation of biofertilizer with chemical fertilizer for the next 15 days of treatment:

Treatments	Parameters in cm				
	Number of leaves / Plant	Length of the leaves	Breadth of the leaves	Shoot length	Plant height
Control (without fertilizer)	4.8	4	4	8	9.1
Azospirillum	5.5	5	4.8	9.2	10.5
Phosphate solubilizing bacteria	5.2	5.1	4.3	9.1	10.3
Chemical fertilizer (urea)	5.4	4.8	4	9	10
Azospirillum + Phosphate solubilizing bacteria (Azo + PSB)	7.5	6.1	5.2	10.2	11.3
Azospirillum + phosphate solubilizing bacteria+chemical fertilizer(urea)(Azo+PSB+CF)	7.3	6	5	10	11

Table 3

Values are expressed in mean ±

This table showed the mean of number of leaves, length and breadth of the leaves (leaf area), shoot length and plant height for the second 15 days of the treatment. The mean of number of leaves, length and breadth of the leaves (leaf area), stem length, plant height was higher in *Azospirillum* and Phosphate solubilizing bacteria combined inoculation. There was no significant difference in the combined inoculation of *Azospirillum* + Phosphate solubilizing bacteria and mixed inoculation of *Azospirillum* + Phosphate solubilizing bacteria + Chemical fertilizer. A low effects were observed in treatments of *Azospirillium* alone, Phosphate solubilizing bacteria alone, Chemical fertilizer (urea) alone and control.

Table 4: Effect on morphological parameters of lady's finger (*Abelmoschus esculentus*) plants inoculated with bacterial biofertilzer alone, chemical fertilizer alone, combined inoculation of biofertilizers and mixed inoculation of biofertilizer with chemical fertilizer for third 15 days of treatment:

Treatments	**Parameters in cm**				
	Number of leaves/ plant	Length of leaves	Breadth of leaves	Shoot length	Plant height
Control (without fertilizer)	4.5	4.3	4	8	9
Azospirilum	5.5	4.3	4.5	10	11
Posphate solubilizing bacteria	5.3	4.3	4.2	9.8	11.1
Chemical fertilizer (urea)	5	4.2	4.1	8	10
Azospirillum+Phosphate solubilizing bacteria (Azo+PSB)	7.7	6.2	5.5	12.5	12.5
Azospirillum+phosphate solubilizingbacteria + chemical fertilizer(urea)(Azo+PSB+CF)	7.4	6.1	5.3	12.0	12.3

Table 4

Values are expressed in mean ±

This table showed the mean of number of leaves, length and breadth of the leaves (leaf area), shoot length and plant height for the third 15 days of the treatment. The mean of number of leaves, length and breadth of the leaves (leaf area), stem length, plant height were higher in *Azospirillum* and Phosphate solubilizing bacteria combined inoculation. There was a no significant difference in combined inoculation of *Azospirillum* + Phosphate solubilizing Bacteria and mixed inoculation of *Azospirillum* + Phosphate Solubilizing bacteria + Chemical fertilizer. A low effects were observed in treatments of *Azospirillium* alone, Phosphate solubilizing bacteria alone, Chemical fertilizer (urea) alone and control.

Table 5: Effect on morphological parameters of lady's finger (*Abelmoschus esculentus*) plants inoculated with bacterial biofertilzer alone, chemical fertilizer alone, combined inoculation of biofertilizers and mixed inoculation of biofertilizer with chemical fertilizer for 60 days of treatment:

Treatments	**Parameters in cm**		
	Root length	Dry weight	Number of pods
Control (without fertilizer)	8	0.15	1
Azospirillum	9.84	0.27	1.2
Phosphatesolubilizing bacteria	9.45	0.25	1.5
Chemical fertilizer(urea)	10	0.20	2.0
Azospirillum + Phosphate solubilizing bacteria (Azo + PSB)	10.54	0.60	4.5
Azospirillum+phosphate solubilizingbacteria+ chemical (Azo + PSB + CF)	10.1	0.30	3.0

Table 5

This table showed the mean of root length, dry weight and number of pods for the 60 days of the treatment. The mean of root length, dry weight and number of pods were higher in *Azospirillum* and Phosphate solubilizing bacteria combined inoculation. There was a no significant difference in the combined inoculation of *Azospirillum* + Phosphate solubilizing bacteria and mixed inoculation of *Azospirillum* + Phosphate solubilizing bacteria + Chemical fertilizer. A low effect was observed in various alone treatments of *Azospirillium* alone, Phosphate solubilizing bacteria alone, Chemical fertilizer (urea) alone and control.

4. Discussion

4.1. Soil Characteristics

The results showed that the pre-cropping analysis of the soil samples were alkaline in nature with adequate amounts of N, P and K. After cropping (biofertilizer inoculation) the soil samples used showed that nitrogen and potassium levels were decreased, whereas phosphate levels were slightly increased showed the uptake of nutrients, nitrogen and potassium by the assimilation of plants. The phosphate in soils was immobilized or becomes less soluble either by absorption, chemical precipitation, or both (Tilak *et al.* , 2005). Nitrogen typically made up around 4% of the dry weight of plant matter (http: //: wikipedia).

The effect of fertilizers on vegetative growth of lady's finger was significantly higher in the combined inoculation of A*zospirillium* and phosphate solubilizing bacteria than control plants.

4.2. Number of Leaves

The maximum number of leaves was found in the combined inoculation of *Azospirillum* and Phosphate solubilizing bacteria (22.6). Leaf production was enhanced significantly by multiple nutrients integration. Reasons for such prolonged leaf production and delay leaf shedding in *Azospirillum* inoculated plants may be due to production of phytohormones like indole acetic acid, gibberellins and cytokines as reported under in- vitro conditions by Bhaskara Rao and Charyulu, (2005). The similar result of the study was well agreed with previous finding of Senthil Kumar and Sivagurunathan (2012).

4.3. Leaf Area / Size

Then the maximum leaf area was observed in the combined inoculation of *Azospirillum* and phosphate solubilizing bacteria (17.4 and 16.6). A similar finding was observed by Panwar *et al.,* (2000). Increases in leaf area index were observed in different crops inoculated with *Pseudomonas*, *Azospirillum* and *Azotobacter* strains by Siddiqui (2002) and Shaukat *et al*., (2006). The obtained results for leaf area might be attributed to nitrogen for improving growth and hence increased leaf area that intercept light radiation and increased photosynthetic rates resulting in accumulation of dry matter Rekhi *et al* ., (2000).

4.4. Shoot Length and Plant Height

Then maximum shoot length and plant height was observed in combined in inoculation of *Azospirillum* and phosphate solubilizing bacteria (31.7and 34.3). The similar finding was observed in Lady's finger by Arangarasan *et al*., (1998) and Chendrayan *et al*., (2003). Dual inoculation with *Azospirillium* and Phosphate solubilizing bacteria resulted in maximum shoot length. The similar finding was observed by Khan *et al.* (2009) stated that maximum growth in biofertilizer treated plant was mainly due to the ability to solubilize phosphate and to produce siderophores and hormones. The enhanced growth and yield of the plants in response might be due to augmentation of nutrients (N and growth factors like IAA) by *Azospirillum* coupled with phosphorus made available by phosphate-solubilizing bacteria which are in agreement with those reported in pearl millet. Phosphate solubilizing bacteria stimulated the growth of shoots and increased phosphate uptake in *Canola* Lifshitz *et al* ., (1987).

4.5. Root Length

Then maximum root length was observed in the combined inoculation of *Azospirillum* and phosphate solubilizing bacteria (10.54). The beneficial effect of *Azospirillum* might derive nitrogen fixation and stimulate root development (Wua *et al*., 2004). This finding was agreed with Noshin and Sumera (2008). Phosphate solubilizing bacteria stimulated the root development and increased phosphate uptake in *Canola* Lifshitz *et al .,* (1987).

4.6. Dry Weight

Then the maximum dry weight was observed in the combined inoculation of *Azospirillum* and phosphate solubilizing bacteria (0.60). Phosphorus besides to nitrogen was one of the most important elements in the crop production. Das *et al* (1997) observed 4.2 to 6.1 % increased in dry matter yield in three varieties of sorghum inoculated with *Azospirillium.* Sutaliya and Singh (2005) also reported dry matter in maize due to Phosphate solubilizing bacteria inoculation.

4.7. Number of Pods

Then maximum number of pods were observed in the combined inoculation of *Azospirillum* and phosphate solubilizing bacteria (4.5). Ponnuswamy *et al*., (2002) reported that a combination of phosphobacteria and *Azospoirillum* had a positive effect on yield and yield characters. Increased grain yield in *Oryza sativa* upon dual inoculation with *Azospirillum* and phosphate solubilizing bacteria also reported by Vendan and Subramanian (1998). Guggari and Kalaghatagi (2005) also observed higher grain yield in peal millet with combined inoculation of *Azospirillum* and phosphate solubilizing bacteria.

5. Conclusion

The results of the study conclude that the number of leaves, length and breadth of the leaves (leaf area), shoot length, plant height, root length, dry weight and number of pods were increased in co-inoculation of *Azospirillum* and Phosphate solubilizing bacteria when compared with other treatments and control. But there was no significant difference with the mixed inoculation of *Azospirillum*, phosphate solubilizing bacteria and chemical fertilizer (urea). A low results were observed in the various alone of *Azospirillum*, phosphate solubilizing bacteria and chemical fertilizer (urea). The control also had a low result. This might be due to the uptake of both nitrogen and phosphorus by *Azospirillum* and phosphate solubilizing bacteria to the plant.

The lights of the results achieved from the study concluded that using combined inoculation of biofertilizers had a maximum impact on soil improvement, plant growth and yield.

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