EFFECT OF BIOFERTILIZERS IN GERMINATION AND BIOCHEMICAL CONTENT OF *ORYZA SATIVA* L. VARIETY AMBAI-16

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Abstract :In Modern agriculture, use of chemical is essential for sustainable yield but these are not eco friendly. Indiscriminate use of chemical fertilizers has developed disturbances in the soil reaction, development of nutritional imbalance in plants, increased susceptibility to pests and diseases. The problem is so intensive that, in many agricultural land of our country less than one crore of micro organisms have been found in one gramof soil and this produce polluted soil which cause environments destabilization making the cost production high. This study proved that combined effect of biofertilizers increased the biochemical and germination percentage in *Oryza sativa* L. variety Ambai-16.

Key words : Biofertilizers, eco-friendly, chemical fertilizers.

INTRODUCTION

India, the second largest population country, mostly depends on agriculture for living. Agriculture primarily depends on soil, which is a living body, because it consists of micro flora such as bacteria, actinomycetes, fungi and algae. In tropical and subtropical agricultural land, normally there are ten crores of microorganisms in one gram of soil. Among the ten crores of microorganisms, only 5-7% is harmful, while the rest are beneficial in nature and extremelyusefulin agriculture (Chowdhury and Mukherjee, 2006).

In modern agriculture, use of chemical fertilizers is essential for sustainable yield but these are not ecofriendly. The chemical fertilizers pose a health hazard and microbial population problem in soil besides being quite expensive and making the cost of production high (Chandrasekar *et al*., 2005).

Indiscriminate use of chemical fertilizers has developed disturbances in the soil reaction, development of nutrient imbalances in plants, increased susceptibility to pests and diseases, reduction in legume root nodulation and plant mycorrhizal associations, decrease in soil life and environmental hazards such as water pollution and soil humus reduction. One of the major effects of such activities is gradual decrease in the number of useful microorganisms in agricultural soil. The problem is so intensive that, in many agricultural land of our country less than one crore of microorganisms has been found in one gram of soil (Chowdhury and Mukherjee, 2006). Because of this reasons, not only the soil is polluted through environment destabilization but the yield of agricultural produce also fluctuating alarmingly. In such a situation biofertilizers play a major role. Authors have reported that biofertilizers harvest atmospheric nitrogen and make it available directly to the plant (Ganachitra, 2000).

Plant Materials

Biofertilizersused in the experiments were *Azospirillum, Mycorrhizae* and *Phosphobacterium*.O*ryza sativa* L. Var. Ambai-16 was taken for investigation.

Sample collection and soil nutrient analysis

Collection of seeds

The seeds of *Oryza sativa* L. Var. Ambai-16 was obtained from Tamil Nadu Agricultural University, Coimbatore.

Collection of biofertilizers

The biofertilizers selected for the study were (*Azospirillum, Mycorrhizae and Phosphobacterium*) collected from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Pesticide treatment

The viable seeds were surface sterilized with 0.1% mercuric chloride for 1-2 minutes and then repeatedly washed with sterilized distilled water to remove any trace of mercuric chloride.

Soil analysis

Soil samples were collected from the experimental field and analysed in soil testing laboratory for nitrogen, phosphorus and potassium (Black, 1957).

The pH of the soil was determined using pH meter (Systronic model 321) and the mean values were calculated.

Experimentation

The treated seeds were sown in eight experimental pots .One of the pot was used control without any fertilizers. The remaining seven pots were treated as experimental pots in which 50 gm of seeds inoculated with biofertilizers (Mono, dual and combined types). Irrigation was done at regular intervals without causing any physical damages.

The experiment was started from the appearance of the third leaf stage which is the experimental stage and it was completed till the senescence of 3rd leaf. The experimental were repeated three times. The different inoculations were as follows:Control, *Azospirillum, Mycorrhizae, Phosphobacterium, Azospirillum + Mycorrhizae, Azospirillum + Phosphobacterium, Mycorrhizae + Phosphobacterium, Azospirillum + Mycorrhizae + Phosphobacterium*

Germination percentage

The number of seeds germinated, were counted to find out the germination percentage. It was calculated the formula

$$\text{Germination Rate} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

The germination rate is expressed in Percentage (%).

Experiments were conducted after the formation of the third leaf (0 day) up to senescence stage.

Biochemical studies

Extraction and estimation of Chlorophyll and Carotenoid were done by Arnon, 1949 method.

RESULTS

Seed germination (*Oryza sativa* L. Var. Ambai-16)

The result on the seed germination percentage of *Oryza sativa* L. Var. Ambai-16 was presented in Table 1.

The control showed the lowest seed germination of 63.33± 4.51%. In mono inoculations of biofertilizers treatment the minimum percentage of seed germination was recorded in *Mycorrhizae* (69.00 ± 2.00%).In dual inoculations *Azospirillum±Mycorrhizae* showed minimum germination (73.67 ± 4.04%) and *Azospirillum±Phosphobacterium* showed maximum germination percentage (76.67 ± 4.73%). The combined biofertilizers treatment showed (92.67± 4.04%) maximum percentage of seed germination when compared to all other treatments.

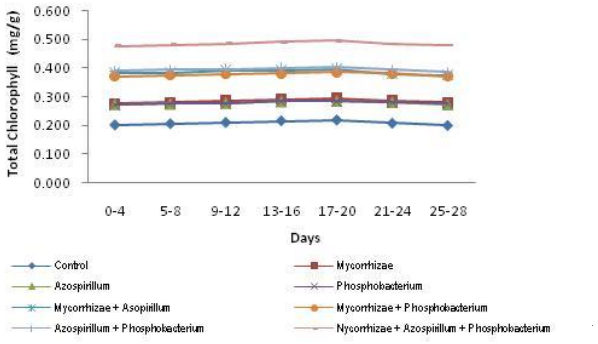
Table 1: Response of biofertilizers on the percentage of seed germination in *Oryza sativa* L. Var. Ambai-16

Biofertilizer	Germination % of seed
Control	63.33± 4.51
Mycorrhizae	69.00 ± 2.00
Azospirillum	72.00 ± 3.61
Phosphobacterium	72.67 ± 3.06
Mycorrhizae + Azospirillum	73.67 ± 4.04
Mycorrhizae + Phosphobacterium	74.33 ± 4.16
Azospirillum + Phosphobacterium	76.67 ± 4.73
Mycorrhizae + Azospirillum + Phosphobacterium	92.67± 4.04

Total chlorophyll content in *Oryza sativa* Var. Ambai-16

Total chlorophyll gradually increased from 0 to 20th day and then gradually decreased from 21st to 28th day in all inoculations. On the 28th day control showed the total chlorophyll of 0.200±0.001 mg/g. In mono inoculations *Azospirillum* showed 0.273±0.001 mg/g, *Mycorrhizae* showed 0.279±0.001 mg/g and in *Phosphobacterium* the total chlorophyll content was 0.274±0.001 mg/g. In dual inoculations *Azospirillum + Mycorrhizae* showed 0.376±0.001 mg/g, *Azospirillum + Phosphobacterium* showed 0.387±0.001 mg/g and *Mycorrhizae + Phosphobacterium* showed 0.373±0.001 mg/g. The combined inoculation showed the maximum total chlorophyll content of 0.496±0.001 mg/g on 20th day compared to mono and dual inoculations. (Fig.1).

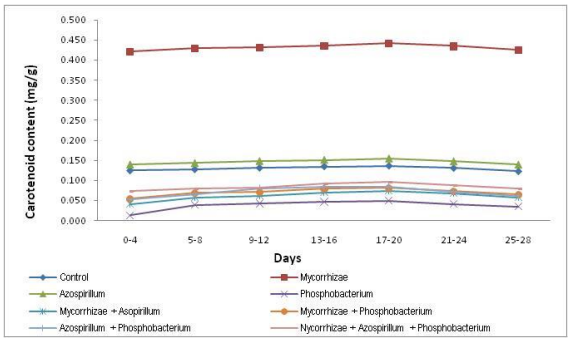
Figure. 1 : Response of biofertilizers on the total chlorophyll content (mg/g) in *Oryza sativa* L. Var. Ambai-16



and then gradually decreased from 21st to 28th day in all inoculations. In mono inoculations maximum carotenoid content (0.441±0.001 mg/g) was found in *Mycorrhizae*. In dual inoculations maximum carotenoid content(0.084±0.001 mg/g) was found in *Phosphobacterium + Azospirillum*.But the combined inoculation showed the maximum carotenoid content of 0.096±0.001 mg/g on 20th day.

On the 28th day control showed the carotenoid content of 0.122±0.001 mg/g. In mono inoculations *Azospirillum* showed 0.139±0.001 mg/g, *Mycorrhizae* showed 0.425±0.002 mg/g and *Phosphobacterium* showed carotenoid content of 0.035±0.001 mg/g on 28th day. In dual inoculations *Azospirillum* + *Mycorrhizae* showed the value of 0.057±0.002 mg/g, *Azospirillum* + *Phosphobacterium* showed 0.062±0.001 mg/g and *Mycorrhiza*e + *Phosphobacterium* showed the carotenoid content of 0.065±0.001 mg/g. Combined inoculation showed highest carotenoid content 0.079±0.001 mg/g on 28th day. (Fig. 2).

Figure. 2: Response of biofertilizers on the carotenoid content (mg/g) in *Oryza sativa* L.Var. Ambai-16



* 0 day = The day of emergence of 3rd leaf

DISCUSSION

Biofertilizers contain microorganisms, the medium through which they are applied in the soil may have significant influence on their effectiveness. Biofertilizers are cheaper, pollution free and renewable in nature (Jain and Singh, 2003).

Biofertilizers are "Microbial inoculants" which fix atmospheric nitrogen and used to improve several crop plants. They are the product containing living cells of different microorganism which have ability to mobilize nutritionally important element from non-usable to usable form through biological process.

Biofertilizers based on renewable energy source are a cost effective supplement to chemical fertilizers and can help to economise on the high investment needed for fertilizer use as far as nitrogen and phosphorus are concerned (Kushare *et al.*, 2009).

Seed germination percentage was investigated in the seeds inoculated with biofertilizers and uninoculated control. Highest germination percentage was found in combined inoculation than in the other inoculations and control. The seed germination of black gram was generally favoured more by biofertilizer than green manure soil amendments (Neelamegam *et al*., 2007).Biofertilizer inoculation influenced the seed germination to an appreciable level compared with control. Highest germination percentage was found in combination for dual inoculation (*Rhizobium* and *Vesicular arbuscular mycorrhiza)* with single super phosphate (SSP) (Pandey *et al*., 2003).

Inoculation of*Vicia faba* with *Mycorrhizae* had positive effect due to stimulatory effect on growth may be due to availability of phosphorus which enhances the metabolic processes such as photosynthesis, starch synthesis, glycolysis and synthesis of fats and proteins (Jan *et al*., 2009). *Mycorrhizal* fungi interact with a wide range of other soil organism in the root, rhizosphere and in the bulk soil. (Boby *et al*., 2007).

Treatments of *Azospirillum* and *Azotobacter* plus 100% Urea resulted in significant increase in total chlorophyll. The chlorophyll content decreased during senescence stage due to the loss of greenness as the plant matures. The beneficial effects of bacterial inoculation on increased chlorophyll content might have been due to the supply of higher amount of nitrogen to the growing tissue and organs supplied by Nitrogen fixing *Azospirillum* and *Azotobacter* (Chandrasekar *et al.*, 2005).

All the inoculated *Chrysanthemum* plants showed superior chlorophyll content compared to control (Singh *et al*., 2008). All *Arbuscular mycorrhizal* fungi inoculation significantly enhanced chlorophyll content by 50% to 60% over control (Ghosh *et al*., 2008).

Carotenoids are the accessory pigments in photosynthetic systems and give characteristic colour to plant parts, particularly flowers and fruits (Sadasivam and Manickam, 2008). Earlier Bahadur *et al.,* (2006) in Broccoli and Chinese cabbage also noticed increased in the carotenoid content respectively with combined use of organic manures and seedling inoculation in *Vesicular arbuscular mycorrhizae* (VAM) or *Azotobacter*.The *arbuscular mycorrhizae* inoculated plants showed significant increase in carotenoid content than the non-mycorrhizal plants (Paneerselvam and Thamizhiniyan, 2008). The combined treatment of micronutrients and *Rhizobium* inoculants increase the carotenoid content in *Glycine max* (Murugesan, 2008). The carotenoid content increased in the tomato plants treated with *Azotobacter* and *Phosphobacterium* (Selvarathi,*et al*., 2010).

Increased carotenoid content under *Azotobacter* and acid content with V*esicular Arbuscular Mycorrhizae* (VAM) may be due to production and synthesis of hormones and vitamins (Vitamin B_{12} and other vitamins) with enhanced enzymatic activity in organic amended and microbial soil of nitrogen (Selvakumar *et al*., 2009).

In the present study, combined inoculation of fertilizers showed more significant result than other inoculations. It is related with the findings of Selvakumar *et al*., 2009.

CONCLUSION

In this investigation the combined effect of *Azospirillum, Phosphobacterium and Mycorrhizae*in *Oryza sativa* is more significant when compared to control and other inoculations.

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