

Impact of Biofertilizers on Physiological characteristics of Rice (*Oryza sativa* L.) cultivar Jaya

*¹N. B. Pawar and ²N. S. Suryawanshi

¹Department of Botany,
Mahatma Phule Arts, Science & Commerce College, Panvel Navi Mumbai,-410206 (India)

²Research Laboratory, Department of Botany,
K. V. Pendharkar College of Arts, Science and Commerce,
Dombivili (E)-421203 Mumbai (India)

*Corresponding Author: nbpawar01@gmail.com

Abstract

Present study was conducted from August 2013 to April 2017 with special emphasis during two kharif seasons (June to November) of 2014-15 and 2015-16. Randomized Block Design (RBD) method was followed to assess the effect of soil based biofertilizers on physiological parameters (photosynthetic pigments such as chlorophyll-a,-b, & total chlorophyll, carbohydrates, crude protein, proline and polyphenol contents) in paddy (*Oryza sativa* L. cv. Jaya) leaves on 30th, 60th and 90th days after transplantation of 21 days old seedlings and before harvesting. RBD techniques was replicated thrice with twelve treatments such as T0: Control (without fertilizer), T1: Chemical fertilizer (19:19:19), T2: Blue Green Algae (BGA), T3: *Azospirillumbrasilense*, T4: *Bacillus megaterium*, T5: *Trichoderma viride*, T6: Mycorrhizae, T7: *Pseudomonas fluorescens*, T8: BGA+*P. fluorescens*, T9: BGA+*Mycorrhizae*, T10: *A. brasilense*+*B. megaterium* and T11: *A. brasilense*+*B. megaterium*+*P. fluorescens*. Results of the study showed that higher values for all physiological parameters were observed for all plants treated with biofertilizers. Also, highest values were recorded in the combined biofertilizer application, than the single or dual combination of biofertilizers on 30th, 60th and 90th days after transplanting (DAT) and before harvesting in the leaves of paddy. This study recommends that, as the biofertilizers are cost effective and environment-friendly, they can be used on large scale to substitute the chemical fertilizers. Similarly, awareness programmes should be organized to create awareness among farmers for application of combination of biofertilizers in intensive agricultural practices. Government officials also enhance the use of biofertilizers by the farmers by providing subsidies on their prices and make them easily available to the farmers to their nearest station or local market.

Key words : Biofertilizers, Carbohydrates, Chlorophyll, *Oryza sativa*, Polyphenols, Proline, Proteins.

Increasing food demand challenge and lessening water availability has become a threat to Asian food security in which almost sixty percent (60%) of the population around the globe lives in the region^{4,37}. The global population is exploding at an exponential rate and is expected to reach approximately 9.7 billion by 2050. This has imposed a large burden on agriculture and its allied sectors in terms of meeting food demands, which requires more inputs for crop production¹⁸.

Paddy is among the most widely cultivated, used and nutritionally relied crops in the world, supplying food to more than 3 billion people globally. Among cereals, the crop has a prominent position and is popularly used worldwide as staple and food which has significant role in addressing food needs. Due to its major contributions in the past decades to hunger reduction and starvation in many parts of the world, significant efforts have been made to raise its production²⁰.

Wencheng *et al.*,⁴⁰ stated that, rice (*Oryza sativa* L.) is one of the most important food crops considered as a major source of calories for more than half of the global population and covers 11% of total arable land⁵. Rice is the most widely cultivated crop and the longest cultivated cereal in the world. It plays a vital role in human food as well as nutritional security for millions of livelihoods. Therefore, the slogan "Rice is life" seems to be most appropriate²².

Around 90% of the total rice in the world is produced and consumed in Asia. More than 50% of the world's population that resides here depends mainly on rice as staple food¹⁷. In Asia, India has the largest area under rice

(41.66 million ha) accounting for 29.4 per cent of the global rice area and is a staple food crop of 63 to 65% people of India³⁴. It is the staple food for more than 70% of Indian, which is grown in 44 million hectares with a production of about 90 million tons. It is estimated that rice demand in 2025 will be 140 million tons in India^{6,39}.

According to Singh *et al.*,³⁵ at present, the increasing productivity of rice is related to the increased application of chemical fertilizer. Due to global environmental changes and crises, massive application of chemical fertilizers, is certainly a key limiting factor. Therefore, it is essential to develop and adopt an integrated policy of nutritional support to supplement and make sensible application of nitrogen as fertilizers with the addition of suitable environment friendly alternative resources¹⁷. Applying alternative fertilization practices instead of conventional fertilizers might improve rice yield and nutrient use efficiency in rice cropping systems⁴⁰.

For paddy, the important yield limiting factors are the water and mineral stress, diseases, insect pest, and weeds. To improve and/or stabilize yield, these yield limiting factors should be managed sustainably²². Deficiencies, excesses or imbalances of various nutrients are known to result in disorders that can limit the quality of the crops. Though chemical fertilizers nourish plants, they also jeopardize the environment through nitrate pollution and create adverse effects on the fragile ecosystem with elimination of beneficial soil organisms and deterioration of physical and chemical properties of soil²¹.

Ojha *et al.*,²⁶ stated that, biofertilizers are preparations containing living cells or latent cells of efficient strains of microorganisms that

help crop plants' uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil. They accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants. Use of biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture³⁷.

Ghimire *et al.*,¹² reported that, biofertilizers can be grouped on their nature and function, such as: Nitrogen fixing (*Azotobacter*, *Beijerinckia*, *Clostridium*, *Klebsiella*, *Anabaena*, *Nostoc*, *Rhizobium*, *Frankia*, *Anabaena azollae*, & *Azospirillum*); Phosphorous solubilizing (Bacteria - *Bacillus megaterium var. phosphaticum*, *Bacillus subtilis*, *Bacillus circulans*, *Pseudomonas striata* & Fungi - *Penicillium* sp., *Aspergillus awamori*); Phosphorous mobilizing (*Glomus* sp., *Gigasporasp.*, *Acaulospora* sp., *Scutellospora* sp. & *Sclerocystis* sp., *Laccaria* sp., *Pisolithus* sp., *Boletus* sp., *Amanitasp.*, *Pezizellaericae*, & *Rhizoctonia solani*); Biofertilizers for Micronutrients (Silicate and Zinc solubilizers - *Bacillus* sp.); and Plant Growth Promoting Rhizobacteria (*Pseudomonas-Pseudomonas fluorescens*)²⁸.

Rana and Kapoor²⁹ argued that biofertilizers/microbial inoculants are used for growing good quality produce and are capable of stopping important nutritional elements in the soil from non-usable to usable form by the crop plants through their biological processes. They are low cost, renewable sources of plant nutrients which supplement chemical fertilizers. Use of Biofertilizer is of great importance because they are components of integrated

nutrient management, and help in reducing the use of chemical fertilizers for sustainable agriculture¹⁰. Biofertilizers can be a supplementary nutrient source for sustainable rice production. It maintains soil fertility, physical, chemical, and biological properties^{12,31}.

Singh *et al.*,³⁴ noted that, biofertilizers are an alternate low-cost resource have gained prime importance in recent decades and play a vital role in maintaining long term soil fertility and sustainability. They are cost effective, eco-friendly and renewable sources of plant nutrients to supplement chemical fertilizers. The application of biofertilizer is cheaper than the inorganic fertilizers. The biofertilizer do not cause damage to the soil and environment like inorganic fertilizers. *Rhizobium*, *Azotobacter*, *Azospirillum* and *Pseudomonas fluorescens* BGA have been in use a long time²⁶.

Chunthaburee *et al.*,⁷ argued that, information on physiological characters may be used to assist in the evaluation of relative field performance of different rice genotypes and characterization of contributing physiological traits that may be employed as reliable indicators for breeding and selection for salt tolerance. Timung *et al.*,³⁹ reported that, assessment of different physiological parameters of rice is important to understand adaptations of the plants to various limiting factors, particularly the water stress as it affects the growth and development. Information on physiological characters plays a vital role in rice breeding and to know the physiological behaviour, genetic expression, and the varietal development programmes^{13,25}.

Further, it was noticed that, worldwide many investigators have studied various aspects of physiological parameters of rice. Work of following investigators is worth to

mention: Thakur *et al.*,³⁸, Doni *et al.*,⁹, Guimaraes *et al.*,¹⁴, Hossain *et al.*,¹⁵, Mishra *et al.*,²³, Sarker *et al.*,³², Sureshkumar and Pandian³⁶, Aswathy *et al.*,², Ichsan *et al.*,¹⁶, Mudoj and Das²⁴, Selvakumar *et al.*,³³, Doni *et al.*,⁹, and Xin *et al.*,⁴¹.

Meagre information is available on the impact of biofertilizers on physiological characters of the rice (*Oryza sativa* L.) from the Konkan region of Maharashtra, west coast of India. Hence the present work would give an account of physiological performance of rice and better orientation towards physio-chemical traits.

Study Area :

The experiments were conducted over two years (2014-15 and 2015-16) at the Department of Botany and Research farm of Rayat Shikshan Sanstha's Mahatma Phule Arts, Science & Commerce College, Panvel, Dist.- Raigad, Navi Mumbai, Maharashtra, India (Lat 18° 59'40" E & 73° 06'50" N) during kharif season. The experimental site was located at Lat 18° 59'40" E & 73° 06'50" N and elevation of about 800 m (2.625 feet) above sea level (Fig. 1).

Collection of biofertilizers :

Biofertilizers such as *Azospirillum brasilense* (Agrosun), *Bacillus megaterium* (Biostila), Blue Green Algae (BGA), Mycorrhizae (Reap Mycorrhiza), *Pseudomonas fluorescens* (Remonas) and *Trichoderma viride* (Bhparistricho) were purchased from Agharkar Research Institute, Gopal Ganesh Agarkar Road, Pune, Maharashtra. The chemical fertilizer (19:19:19-Paras) was purchased from authorized private Agro Centre, Panvel. Effect of biofertilizers on germination and seedling growth of rice were studied using seed treatment, seedling root dip method and Random Block Design (RBD) method.

Carriers for soil inoculation with Biofertilizers: (FNCA, 2006b) :

For soil inoculation, carrier material with granular form (0.5-1.5 mm) was used. Rhizobium and *Aspergillus niger* were used as it survives in dry granules beyond 180 days and never lose its viability in a dry state. Soil was also mixed with soil aggregate and charcoal to provide nutrient and/or habitable micro-pore habitat for soil inoculant.

Combination of biofertilizers for treatment:

Table-1. Combination of biofertilizers used for treatment of rice seeds

Tray No.	Combination of biofertilizers	Concentration
T0	Control	
T1	Chemical fertilizer (19:19:19)	50 kg ha ⁻¹
T2	Blue Green Algae (BGA)	10 kg ha ⁻¹
T3	<i>Azospirillum brasilense</i>	2 kg ha ⁻¹
T4	<i>Bacillus megaterium</i>	2 kg ha ⁻¹
T5	<i>Trichoderma viride</i>	2 kg ha ⁻¹
T6	Mycorrhizae	2 kg ha ⁻¹
T7	<i>Pseudomonas fluorescens</i>	2 kg ha ⁻¹
T8	BGA + <i>Pseudomonas fluorescens</i>	4 kg ha ⁻¹
T9	BGA + Mycorrhizae	4 kg ha ⁻¹
T10	<i>Azospirillum brasilense</i> + <i>Bacillus megaterium</i>	4 kg ha ⁻¹
T11	<i>Azospirillum brasilense</i> + <i>Bacillus megaterium</i> + <i>Pseudomonas fluorescens</i>	6 kg ha ⁻¹

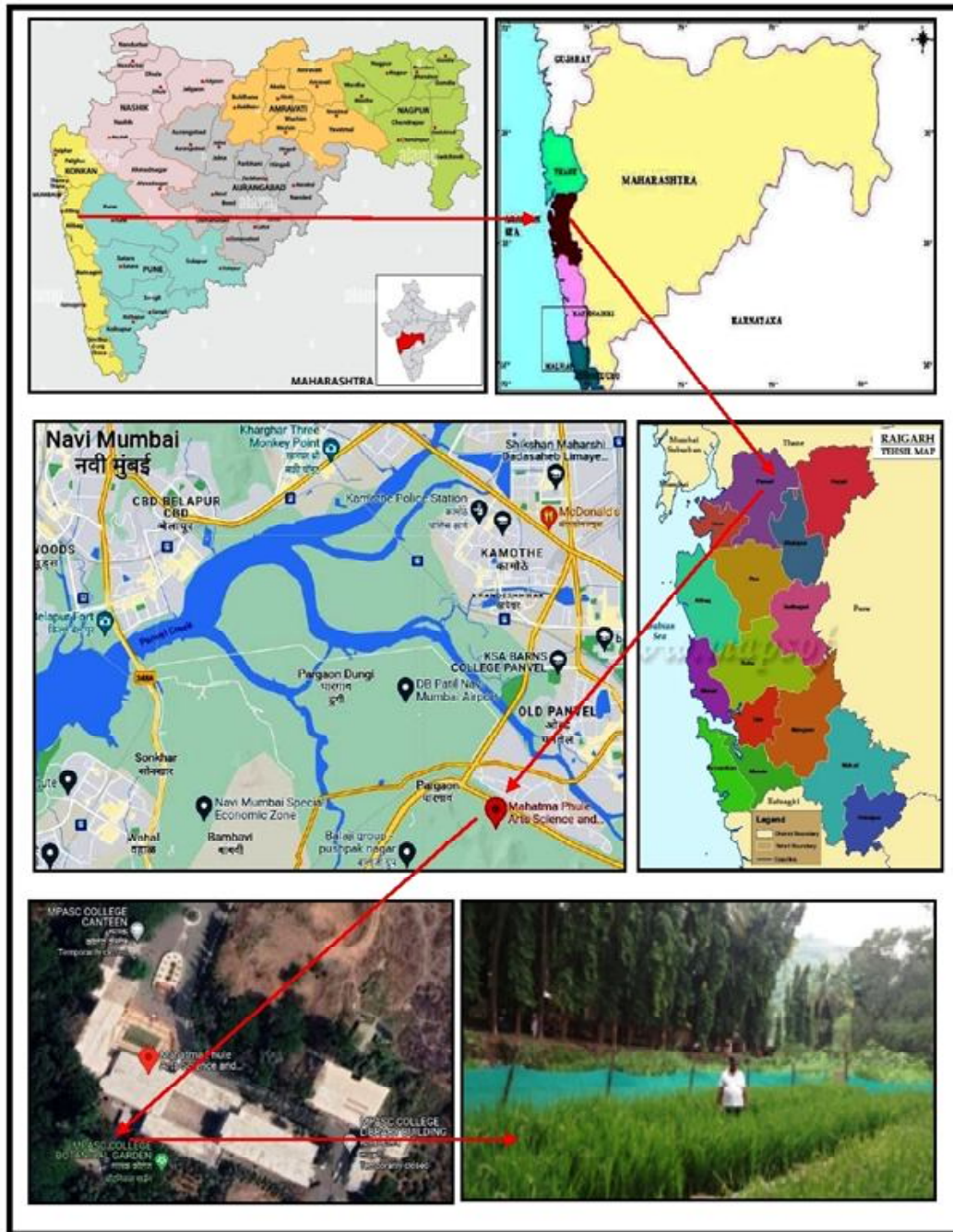


Fig. 1. Location Map of Experimental Site (Source: Google Map)
(Research field at Mahatma Phule Arts, Science & Commerce College, Panvel)

Preparation of nursery plot by culture of seedling :

The rice nursery was cultured in a separate plot in the agricultural field of nearby farmer. The culture bed is with 12 m x 1 m in size and were prepared in a well tilled plot. The nursery plot was irrigated 12 days prior to sowing of seeds and to hasten germination of kharif annual weeds. The plot was ploughed for upturning the soil and was followed by the cross plough with the cultivator.

Good quality seeds of variety Jaya, were treated with the fungicide, Thirum @ 3 g/kg of seed. Seeds were soaked in water for 12 hrs and stored in wet gunny bags in dark for 24 hrs to hasten sprouting. The sprouted seeds were down by broadcasting. Germinated seeds were watered regularly to develop into quality seedlings. All recommended practices were followed in order to ensure healthy growth of seedlings.

Transplantation of Seedlings :

An experimental plot was arranged in random block design with three replicates. The seedlings were uprooted from the nursery bed on the day of transplanting. Twenty-one days

old paddy seedlings were transplanted at 20 cm x 15 cm spacing with five seedlings per hill. Gap filling was carried out ten days after transplanting in order to ensure uniform plant population.

Before transplantation, first dose of chemical fertilizer (NPK - 19:19:19) were applied to T1 sub-pot as basal application. Second dose of chemical fertilizer was applied in two split doses, at tillering and panicle initiation. Recommended dose of soil-based inoculants was broadcasted to standing water in T2 subplot after 5th day of transplantation. During transplantation, mixture of biofertilizers were incorporated in the soil of respective subplot as per recommendations. Plants without treatment of biofertilizers were considered as control (T0). Gap filling was carried out ten days after transplantation in order to ensure uniform plant population. Effect of biofertilizer treatments on the growth and development parameters of paddy was noted during pre-harvest period at 30th, 60th and 90th Days After Transplantation (DAT). During course of present study all the data were analysed by using statistical procedures as described by Panse and Sukhatme²⁷.

Standard Operating Procedures adopted :

Table-2. Standard methods adopted for physiological parameters

Physiological parameters (mg/g)	Standard Operating Procedure (30, 60 & 90 day after Sowing & Before Harvesting)
Chlorophyll (a, b & total chlorophyll)	Arnon (1949)
Total carbohydrates	Anthrone method (Sadasivam & Manickam, 2004)
Protein contents	Lowry <i>et al.</i> , (1951)
Proline contents	Bates <i>et. al.</i> , (1973)
Polyphenols contents	Folin, Denis (1915)

Results on effect of biofertilizer treatment on physiological parameters at various growth stages of paddy by RBD method is presented in Table-3 to 9, and Fig. No. 2 to 8.

Photosynthetic Pigments :

Chlorophyll-a, b, and total chlorophyll contents from control (T0) and experimental leaves from T1 to T11 at 30th, 60th & 90th DAT and before harvesting were assessed to study the biochemical changes in physiologically active leaves of paddy (*Oryza sativa* L.) variety Jaya, under different treatments of biofertilizers.

Chlorophyll-a (Table 3 & Fig. 2) :

It was observed that chlorophyll-a content increased considerably from 30th, 60th & 90th DAT and thereafter, it gradually declines from 90th to harvest stage. Treatment T10 (*A. brasilense* + *B. megaterium*) show significant increase in chlorophyll-a content with moderately high values than T8, T9, and T11 treatments at 30th and 60th DAT (2.11, 2.13 and 2.12 mg/g-1 at 30th DAP & 2.20, 2.34 and 2.27 mg g-1 at 60th DAT) during 2014, 2015 and on pooled data respectively. Values of chlorophyll-a slightly decline at 90th DAT and harvesting stage in T10 T10 (*A. brasilense* + *B. megaterium*), (2.05, 2.08 and 2.07 mg g⁻¹ at 90th DAP and 1.92, 1.85 and 1.88 mg g⁻¹ at harvesting) in both the years and in pooled results.

From single inoculation, higher values were noted in T3 (1.92, 1.98 and 1.95 mg g⁻¹

at 30th DAT and 2.08, 2.12 and 2.10 mg g⁻¹ at 60th DAT) in both years and in pooled mean, and was followed by T5 and T2. At 90th DAT and harvesting, amount of chlorophyll-a slightly declines. Treatment T1 (chemical fertilizer) showed higher values of chlorophyll-a as compared to control (T0) during the both the years and in pooled finding.

Chlorophyll-b (Table 4& Fig. 3) :

At 30th DAT, dual application of biofertilizer, T10 (*A. brasilense* + *B. megaterium*) exhibit maximum value of leaf chlorophyll-b (1.07 mg g-1) and remain at par with other treatments viz. T11, T9, T6, T8, T7, T3, T2, T4, and T5. During 2015, combined biofertilizer treatment, T11 (*A. brasilense*+*B. megaterium* + *P. fluorescens*) recorded highest amount of chlorophyll-b (1.22 mg g⁻¹) and remained at par with treatments T10, T9, T7, T8, T6, T2, T4, T5 and T3. The pooled analysis showed that combined effect of biofertilizer treatment T11 have recorded high amount of chlorophyll-b content (1.14 mg g⁻¹) and remained at par with treatments T10, T6, T8, T7, T2, T4, T5 and T3.

For 60th DAT, during 2014, for treatment T11, highest amount of chlorophyll-b (1.297 mg g⁻¹) recorded and was followed by dual biofertilizer treatments, T10 and T9. Among single inoculation biofertilizer treatments, T3 show maximum value 1.146 mg g⁻¹ of chlorophyll-b and remained at par with treatments T5, T2, T6, T4, T7 and T5. During 2015, treatment T9 (BGA + Mycorrhizae) recorded higher value, 1.67 mg g⁻¹ of chlorophyll-b and remained at par with treatments T10, T11, T8, T3, T7, T6, T4 and

T5. In pooled analysis, higher value of chlorophyll-b (1.47 mg g^{-1}) was noted with T9 treatment of dual inoculation and it remains at par with treatments viz. T10, T11, T3, T8, T6, T2, T7 and T4.

For 90th DAT, during 2014, T11 treatment have recorded maximum value of chlorophyll-b (1.00 mg g^{-1}) over other treatments. Treatment T9 ranked second (0.99 mg g^{-1}) for chlorophyll-b content and remained at par with T10, T2, T3, T4, T6, T5 and T7. During 2015, T10 show high chlorophyll-b pigment (1.15 mg g^{-1}) and remained at par with T11, T9, T8, T6, T4, T7, and T5 for treatment with various biofertilizers. In pooled analysis, for treatment T11, maximum value of 1.07 mg g^{-1} was recorded and remained at par with treatments T10, T9, T8, T2, T6, T3, T4, T7 and T5.

At harvesting, during 2014, treatment T11 have recorded highest value (0.75 mg g^{-1}), followed by T10, T8 & T9 treatments. Among single biofertilizer treatment, T6 ranked first with 0.38 mg g^{-1} and remained at par with T7, T3, T5, T4 and T2. During 2015, T8 show high amount of chlorophyll-b (0.77 mg g^{-1}) and remained at par with T11, T10, T9, T6, T2, T7, T5, T3 and T4 treatments. In pooled analysis, T11 has recorded highest amount of chlorophyll-b (0.75 mg g^{-1}) and remained at par with T10, T8, T9, T6, T7, T3, T5 and T4.

Treatment T1 increased the content of chlorophyll-b as compared to control (T0), during both years and in pooled finding at 30th, 60th & 90th DAT at harvesting stage. Among all treatments of biofertilizers, combined and

dual biofertilizers show results at par with each other and values of chlorophyll-b contents of single inoculated plants in experimental years and in pooled data.

Total chlorophyll (Table-5 & Fig. 4) :

It is noted that, content of chlorophyll-a, b and total chlorophylls was increased significantly from 30th, 60th & 90th DAT and thereafter, gradual decline was observed from 90th DAT and at harvesting stage. At 30th DAT, combined application of biofertilizer T11 (*A. brasilense*+*B. megaterium* + *P. fluorescens*) showed highest value of total chlorophyll content with 3.23 mg g^{-1} and remained at par with dual and mono inoculant biofertilizer treatments viz. T10, T9, T8, T6, T7, T3, T2, T4 and T5.

During 2015, treatment T11 recorded highest value of total chlorophyll content (3.32 mg g^{-1}) and remained at par with treatments T10, T9, T8, T7, T6, T2, T4 and T3. The pooled data show that treatment T11 show higher quantity of total chlorophyll contents (3.28 mg g^{-1}) and remained at par with treatment T11 and superior over T10, T9, T8, T7, T6, T3, T2 and T4.

For 60th DAT, treatment T11, demonstrate highest amount of total chlorophyll contents (3.52 mg g^{-1}) and remained at par with other treatments. Dual biofertilizer treatment T10, (*A. brasilense* + *B. megaterium*) ranked second and remained at par with treatment T10 and T9. The single biofertilizer treatment T3 (*A. brasilense*) observed maximum amount and was par with T3, T4, T2, T7 and T5. During 2015, T9 (*BGA* + *Mycorrhizae*)

exhibit highest value of 3.71 mg g⁻¹ of total chlorophyll and remained at par with treatments T11, T10, T8, T7, T6, T4, T3 and T2. In pooled analysis, significantly higher value (3.61 mg g⁻¹) was noted with T11 and it remains at par with treatments viz. T9, T10, T8, T3, T6, T7, T2 and T5.

AT 90th DAT, during 2014 and 2015, T11 show higher content of total chlorophyll (3.14 & 3.27 mg g⁻¹) and remained at par with T9, T10, T2, T8, T6, T4, T3, T7 and T5. In pooled analysis, treatment T11 recorded significantly higher value (3.21 mg g⁻¹) and stand superior over the treatments T9, T10, T8, T2, T6, T4, T7, T3 and T5. At harvest, during 2014, T11 record highest value of total chlorophyll content (2.59 mg g⁻¹) than other treatments. T10 ranked second with 2.43 mg g⁻¹ and remained at par with T8, T9, T6, T7, T3, T5, T4 and T2. During 2015, T11 show maximum amount of total chlorophyll (2.62 mg g⁻¹) and remained at par with T10, T9, T8, T2, T7, T3, T5 and T4.

In pooled analysis, T11 record highest total chlorophyll content (2.61 mg g⁻¹) and remained at par with T9 and T8. For mono inoculants, T6 (Mycorrhizae) exhibit higher value (2.25 mg g⁻¹) and it was significantly superior over treatments T7, T3, T5 and T4. T1 show better results for total chlorophyll, as compared to control (T0), during both years and in pooled finding at 30th, 60th & 90th DAT and at harvest. Combined and dual biofertilizer treatments are at par with each other and content of total chlorophyll, was better than mono inoculated, for both and even in pooled data.

Total Carbohydrate contents (Table-6 & Fig. 5):

Treatment with various biofertilizers have significantly influenced the total carbohydrate contents in paddy leaves at various growth stages. For 30th DAT, during 2014, T11 (*A. brasilense* + *B. megaterium* + *P. fluorescens*) have recorded highest value of total carbohydrate content (68.40 mg g⁻¹) and it remain at par with single and dual inoculant biofertilizer treatments viz. T10, T9, T8, T7, T3, T5, T4, T2 and T6. During 2015, these values were 79.35 mg g⁻¹ and remain at par with treatments T10, T9, T6, T8, T2, T3, T4 and T5. Analysis of pooled data show high values for total carbohydrate *i.e.* 73.87 mg g⁻¹ with treatment, T11 and it is greater than treatment with dual biofertilizer (T10, T9 & T8). Among single biofertilizer treatment, T7 (*P. fluorescens*) has recorded highest value of total carbohydrate (66.11 mg g⁻¹) and was at par with T2, T3, T4, T6 and T5.

At 60th DAT, highest total carbohydrate content (119.07 mg g⁻¹) was noticed in T11 and is the highest value recorded from all other treatments. Among dual biofertilizer treatment, T10 (*A. brasilense* + *B. megaterium*) exhibit moderate values and remained at par with treatments T9, T10 and T8. From single biofertilizer treatment, T4 (*B. megaterium*) show higher total carbohydrate contents and was par with T2, T7, T6, T5, and T3. During 2015, highest recorded value for T11 is 129.76 mg g⁻¹ and remain at par with treatments T9, T10, T8, T7, T6, T2, T5, T3 and T4. Further, highest value recorded from pooled analysis for T11 is 124.42 mg g⁻¹ and it remain at par with treatments, T9, T10, T2, T7, T8, T6, T4 and T5.

At 90th DAT, T11 have exhibited highest values for total carbohydrate contents such as 141.94 mg g⁻¹ during 2014 and 157.98 mg g⁻¹ for 2015 and remain at par with T10, T9, T8, T6, T7, T2, T3, T4 & T5 during 2014 and T11, T10, T8, T7, T2, T3, T6, T4 & T5 for 2015 respectively. In pooled analysis, value recorded for T11 is 149.73 mg g⁻¹ and it remain superior over treatments, T9, T8 T7, T6, T2, T3, T4 and T5.

At harvest, T11 have recorded highest values as 179.347 mg g⁻¹ and 80.783 mg g⁻¹ for the year 2014 and 2015 respectively. For dual application of biofertilizers, T10 (*A. brasilense* + *B. megaterium*) represent moderate value (74.61 mg g⁻¹) of total carbohydrate content and remained at par with T9, T8, T6, T3, T2, T5, T4 and T7. In pooled analysis, highest values were recorded for T11 and are found to be over T9, T10 and T8. For treatment with single biofertilizer, T6 (Mycorrhizae) exhibit higher value of 73.37 mg g⁻¹ and stands superior over T7, T3, T2, T4 and T5. Also, moderate values were represented by T1 (chemical fertilizer) and are found to be over T0 (control) during both years and in pooled analysis at 30th, 60th & 90th DAT.

Crude Protein content (Table-7 & Fig. 6):

It was noticed that combined and dual biofertilizer shows mor prominent increase in crude protein content in paddy leaf. For 30th DAT, T11 (*A. brasilense* + *B. megaterium* + *P. fluorescens*) exhibit highest crude protein content (98.51 mg g⁻¹ in 2014 & 129.69 mg g⁻¹ in 2015) and remained at par with single and dual biofertilizer treatments like T10, T9, T8, T6, T4, T2, T3, T7 and T5 in 2014 and T9, T10, T8, T7, T6, T4, T2, T5 and T3 during

2015. In analysis of pooled data, also T11 has recorded highest crude protein content (114.11 mg g⁻¹) and remained far above the dual (T9, T10 & T8) treatments. Of the single biofertilizer treatment, T7 (*P. fluorescens*) show higher crude protein content (105.48 mg g⁻¹) over the T6, T4, T2, T3 and T5.

At 60th DAT, single biofertilizer treatment, T4 (*B. megaterium*) show highest values of crude protein (87.504 mg.g⁻¹), over the treatments T10, T11, T3, T9, T6, T2, T5 and T7. Further, during 2015, T10 recorded highest crude protein content (113.24 mg g⁻¹) and remained at par with treatment T11, T8, T9, T6, T2, T7, T3, T4 and T5. In pooled analysis, T10 also recorded higher values (99.265 mg g⁻¹) over the treatments T11, T9, T4, T3, T8, T6, T2, T7 and T5.

At 90th DAT, T11 displayed highest content of crude protein (98.512 mg g⁻¹ in 2014 & 93.177 mg g⁻¹ in 2015) and remain at par with T10, T9, T8, T6, T4, T2, T4, T7 & T5 in 2014 and T9, T10, T8, T6, T3, T7, T3, T4 & T5 in 2015. of the single biofertilizer treatment, T3 (*A. brasilense*) record highest crude protein content with 84.59 mg g⁻¹ and remained at par with T7, T6, T2, T4 and T5. In pooled analysis, T11 also exhibit higher values (96.69 mg g⁻¹) of crude protein, over the T9, T10, T8, T6, T3, T7, T3, T4 and T5.

At harvest, T11 exhibit highest values for both years i.e. 50.872 mg g⁻¹ in 2014 and 81.495 mg g⁻¹ in 2015. Also, it remains par with the T9, T8, T7, T4, T2, T5, T6 & T3 for 2014 and T8, T9, T10, T7, T2, T5, T6, T3 & T4 in 2015. From application of dual biofertilizers, T10 (*A. brasilense* + *B. megaterium*) show moderate values (49.93 mg

g-1) of total crude protein and remain par with T9, T8, T7, T4, T2, T5, T6 and T3. In pooled analysis, T11 show highest value of crude protein (66.18 mg g-1) and remained at par with T8, T10 and T9. Among single biofertilizers, T7 (*P. fluorescens*) exhibit higher value of crude protein (54.05 mg g-1) and it remain far over T2, T5, T4, T4, T6 and T3.

Chemical fertilizer treatment (T1), show comparatively higher values over the control (T0) during both years and in pooled finding at 30th, 60th & 90th DAT.

Proline content (Table-8& Fig. 7):

Data obtained on proline content indicates that dual and combined biofertilizer treatment enhances it in both years and also in pooled analysis.

At 30th DAT, highest values of proline content were shown by T11 in both years (8.55 mg g-1 in 2014 & 13.70 mg g-1 in 2015). Also, T11 remain at par with T10, T9, T8, T7, T4, T2, T5, T6 & T3 in 2014 and T8, T9, T10, T7, T2, T5, T6, T3 & T4 during 2015. Results on analysis of pooled data show highest proline content in T11 *i.e.* 11.12 mg g-1 in 2014 and is higher than the results of dual biofertilizer treatments (T8 T10 & T9). T7 (*P. fluorescens*) exhibit higher value (9.08 mg g-1) and was par with T2, T5, T4, T6 and T3.

For 60th DAT, T9 (BGA+ Mycorrhizae) have recorded highest proline content (10.34 mg g-1) over all treatments. T11 show moderate values (10.23 mg g-1 in 2014 & 15.943 mg g-1 in 2015) and remained superior to T10, T5, T8, T4, T7, T6, T3 and T2. In pooled analysis, T11 exhibit higher value (13.09 mg g-1) and remain over T9, T10, T8, T7, T3, T6, T4, T5 and T2.

At 90th DAT, highest proline content (14.706 mg g-1 & 19.033 mg g-1) was recorded by T4 (*B. megaterium*) in 2014 and T10 (*A. brasilense* + *B. megaterium*) for 2015 respectively. T4 and T10 remained at par with T10, T11, T3, T9, T6, T2, T5, T7 & T8 for 2014 and T11, T8, T9, T6, T2, T7, T3, T4, T5 & T6 for 2015. In pooled analysis, T11 recorded highest value (16.68 mg g-1) and remain superior to T11, T9, T4, T3, T8, T2, T6, T7 and T5.

For harvest, highest value of proline content was recorded for T11 (16.556 mg g-1 in 2014 & 21.796 mg g-1 in 2015). During 2014, moderate values were recorded by T10 (16.556 mg g-1) and T11 (21.796 mg g-1). Further, during 2015, high values (21.796 mg g-1), were recorded for T11. All other treatments, T10, T9, T8, T6, T4, T2, T3, T7 & T5 and T9, T10, T8, T7, T6, T4, T2, T5 & T3 were show moderate values for 2014 and 2015 respectively. In pooled analysis, highest values were recorded for T11 (19.17 mg g-1) and T7 (*P. fluorescens*) with 17.73 mg g-1). T11 and T7 were remained at par with T9, T10 & T8 along with T6, T4, T2, T3 & T5. Treatment T1 (chemical fertilizer) show higher values in comparison to T0 (control) in both years.

Polyphenol content (Table 9 & Fig. 8):

Results obtained reveal that both dual and combined biofertilizer treatments influence polyphenol content in both years and in pooled analysis. At 30th DAT, T11 have recorded highest values *i.e.* 1.35 mg g-1 for 2014 and 0.94 mg g-1 for 2015. T11 remained at par with T9, T8, T6, T3, T5, T7, T2 & T4 during 2014 and T9, T10, T8, T3, T7, T6, T2, T5 & T4 for 2015. In analysis of pooled data, highest

value was exhibited by T11 (1.14 mg g⁻¹) and T3 (1.05 mg g⁻¹). T11 exhibit values greater than T9, T10 and T8 and T3 represent values higher than T6, T7, T2, T5 and T4.

For 60th DAT, highest values of polyphenol were recorded by T10 (*A. brasilense* + *B. megaterium*) (5.38 mg g⁻¹ in 2014) and by T11 (9.42 mg g⁻¹ in 2015). T10 shows better values than T11, T9, T8, T3, T4, T6, T7, T2 & T5. Values shown by T11 are higher than T9, T10, T8, T6, T5, T3, T7, T4 & T2. In pooled analysis, higher value (7.39 mg g⁻¹) was recorded for T11 and are higher than the values shown by T10, T9, T8, T6, T5, T3, T7, T4 & T2.

At 90th DAT, highest values of polyphenol content were recorded for T11 *i.e.* 12.63 mg g⁻¹ during 2014 and 19.185 mg g⁻¹ during 2015). During 2014 and 2015. From single fertilizer treatment, T5 (*T. viride*) recorded highest content (17.464mg g⁻¹) and

remained at par with T2, T6, T4, T7 & T3. In pooled analysis, T11 represents highest value (15.59 mg g⁻¹) and was superior over T10, T9, T8, T6, T2, T5, T7, T4 & T3.

At harvest, highest values were observed for T11 *i.e.* 22.083 mg g⁻¹ for 2014 and 21.783 mg g⁻¹ for 2015 respectively. Among dual application, for 2014, T10 (*A. brasilense* + *B. megaterium*) show highest value (21.82 mg g⁻¹) and remained at par with T9, T8, T6, T4, T3, T2, T7 & T5. During 2015, T11 remained at par with T10, T9, T8, T7, T6, T5, T2, T3 & T4. In pooled analysis, T11 show highest value of 21.93 mg g⁻¹ and remained at par with T10, T9 & T8. From single inoculants, T7 (*P. fluorescens*) exhibited higher value of 19.09 mg g⁻¹ and it was superior over T2, T3, T5, T4 & T6. Treatment T1 (chemical fertilizer) show higher values in comparison to T0 (control) in both years at 30th, 60th & 90th DAT.

Table-3. Chlorophyll-a contents (mg/g) in Paddy (*Oryza sativa* L. var. *jaya*) at successive growth stages by RBD method

Treatments	Chlorophyll 'a' (mg/g)												
	30 DAT		PD	60 DAT			PD	90 DAT		PD	At harvesting		PD
	2014	2015		2014	2015	2014		2015	2014		2015		
T0- Control	1.577	1.785	1.681	1.893	1.899	1.895	1.727	1.734	1.732	1.429	1.429	1.429	
T1- Chemi. fertili.	1.971	2.047	2.009	2.121	2.134	2.128	2.015	1.955	1.985	1.749	1.816	1.779	
T2- BGA	1.882	1.928	1.905	2.028	2.066	2.047	1.945	1.965	1.953	1.712	1.694	1.703	
T3- <i>Azospirillum</i>	1.918	1.982	1.951	2.081	2.115	2.095	1.793	1.856	1.824	1.741	1.744	1.743	
T4- <i>Bacillus</i>	1.877	1.918	1.897	2.088	2.107	2.098	1.878	1.922	1.906	1.719	1.724	1.722	
T5- <i>Trichoderma</i>	1.891	1.921	1.906	2.063	2.105	2.084	1.852	1.918	1.885	1.711	1.654	1.682	
T6- Mycorrhizae	2.069	2.051	2.062	2.079	2.117	2.098	1.924	1.941	1.931	1.834	1.738	1.786	
T7- <i>Pseudomonas</i>	2.042	2.062	2.052	2.098	2.113	2.105	1.891	1.945	1.916	1.764	1.746	1.755	
T8- T2+ T7	2.083	2.089	2.086	2.179	2.226	2.202	2.002	2.045	2.023	1.845	1.846	1.846	
T9- T2+ T6	2.114	2.115	2.114	2.204	2.411	2.307	2.135	2.112	2.123	1.867	1.843	1.855	
T10- T3+T4	2.109	2.128	2.119	2.201	2.338	2.269	2.051	2.084	2.067	1.916	1.851	1.881	
T11- T3+T4+T7	2.125	2.108	2.117	2.224	2.312	2.268	2.138	2.129	2.133	1.843	1.867	1.855	
SE m ±	0.021	0.012	0.017	0.020	0.066	0.043	0.034	0.022	0.028	0.022	0.026	0.021	
CD at 0.05 %	0.061	0.036	0.049	0.057	0.187	0.122	0.096	0.064	0.083	0.064	0.058	0.061	
C.V.%	0.159	0.092	0.126	0.139	0.442	0.291	0.252	0.168	0.219	0.188	0.172	0.184	

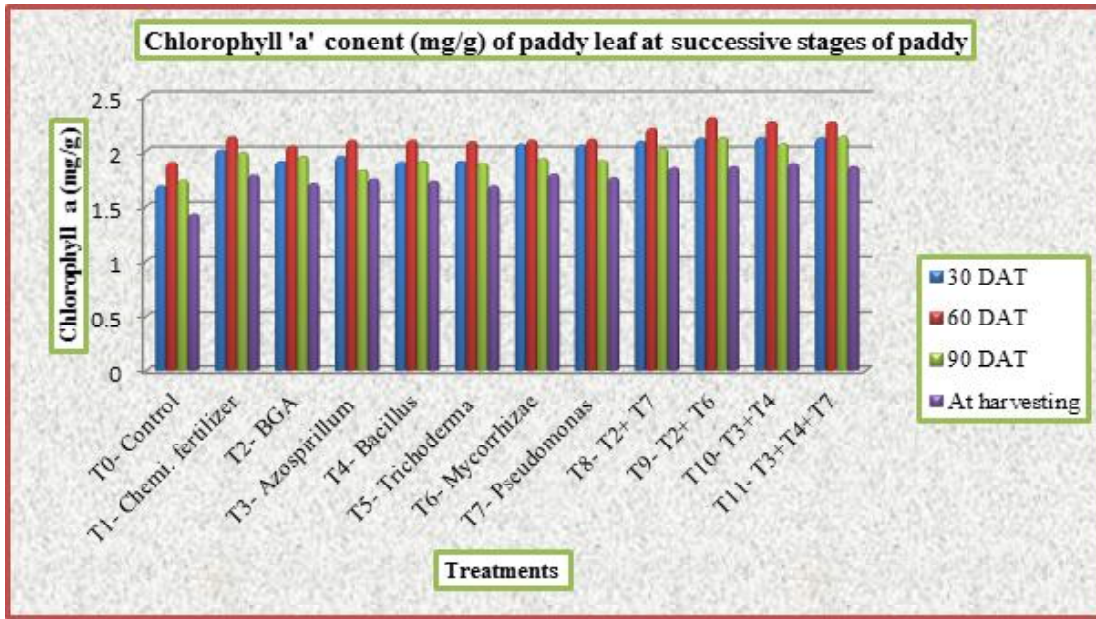


Fig. 2. Effect of biofertilizers on the Chlorophyll-a contents (mg/g) in the fresh leaves of Paddy (*Oryza sativa* L.) variety, Jaya at various stages of its growth by RBD method

Table-4. Chlorophyll-b contents (mg/g) in Paddy (*Oryza sativa* L. var. jaya) at successive growth stages by RBD method

Treatments	Chlorophyll 'b' (mg/g)											
	30 DAT		PD	60 DAT		PD	90 DAT		PD	At harvesting		PD
	2014	2015		2014	2015		2014	2015		2014	2015	
T0- Control	0.674	0.627	0.651	0.763	0.789	0.776	0.531	0.584	0.556	0.313	0.312	0.312
T1- Chemi. fertili.	0.836	0.911	0.873	1.036	1.171	1.103	0.842	0.973	0.907	0.312	0.691	0.501
T2- BGA	0.905	0.925	0.915	1.107	1.129	1.118	0.957	0.853	0.905	0.216	0.503	0.358
T3- Azospirillum	0.906	0.811	0.858	1.146	1.255	1.203	0.901	0.849	0.875	0.307	0.423	0.365
T4- Bacillus	0.878	0.906	0.892	1.048	1.121	1.085	0.875	0.868	0.869	0.221	0.313	0.267
T5- Trichoderma	0.858	0.891	0.874	1.014	1.108	1.061	0.777	0.804	0.791	0.252	0.434	0.343
T6- Mycorrhizae	1.004	1.047	1.025	1.088	1.147	1.118	0.855	0.949	0.902	0.382	0.542	0.462
T7- Pseudomonas	0.919	1.058	0.988	1.031	1.178	1.104	0.756	0.847	0.801	0.357	0.437	0.397
T8- T2+ T7	0.984	1.047	1.015	1.066	1.231	1.148	0.781	1.064	0.922	0.459	0.773	0.614
T9- T2+ T6	1.031	1.126	1.078	1.269	1.669	1.469	0.987	1.135	1.061	0.407	0.715	0.561
T10- T3+T4	1.071	1.152	1.112	1.292	1.452	1.372	0.982	1.152	1.067	0.522	0.719	0.621
T11- T3+T4+T7	1.054	1.216	1.135	1.297	1.386	1.341	1.001	1.143	1.072	0.752	0.757	0.754
SE m ±	0.036	0.033	0.034	0.053	0.107	0.078	0.117	0.059	0.088	0.077	0.087	0.078
CD at 0.05 %	0.103	0.093	0.098	0.143	0.302	0.223	0.331	0.168	0.251	0.219	0.227	0.223
C.V.%	0.568	0.491	0.529	0.668	1.267	0.967	1.984	0.916	1.451	2.988	2.106	2.544

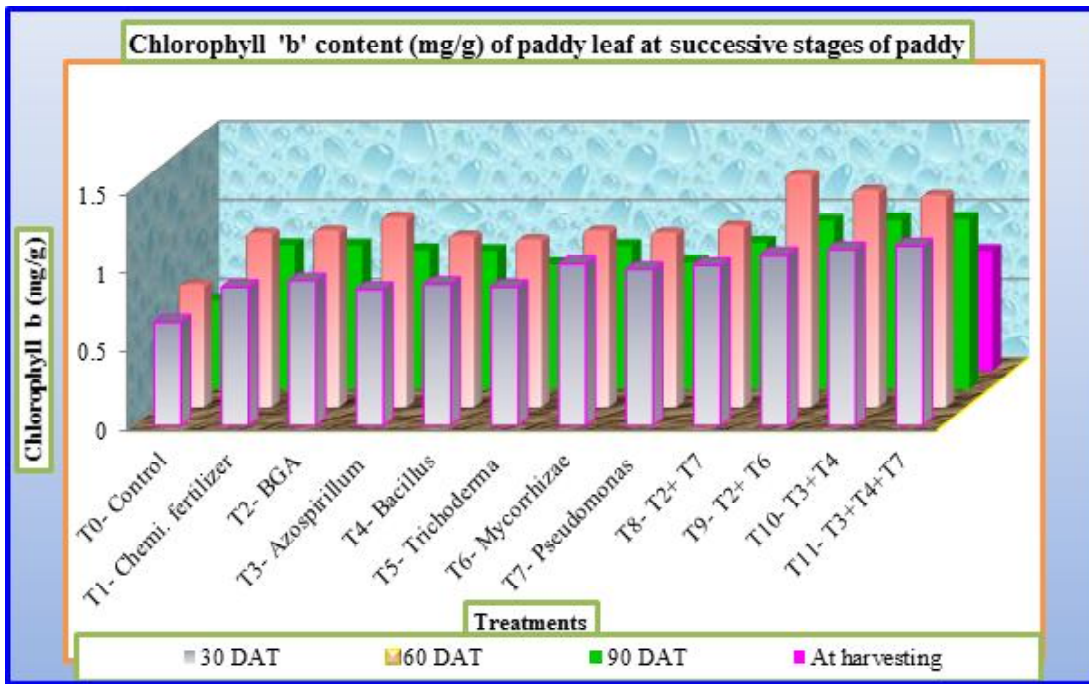


Fig. 3. Effect of biofertilizers on the Chlorophyll-b contents (mg/g) in the fresh leaves of Paddy (*Oryza sativa* L.) variety, Jaya at various stages of its growth by RBD method

Table-5. Total chlorophyll contents (mg/g) in Paddy (*Oryza sativa* L. var. jaya) at successive growth

Treatments	Total Chlorophyll (mg/gm)											
	30 DAT		PD	60 DAT		PD	90 DAT		PD	At harvesting		PD
	2014	2015		2014	2015		2014	2015		2014	2015	
T0- Control	2.251	2.412	2.331	2.653	2.688	2.675	2.258	2.314	2.286	1.428	1.428	1.428
T1- Chemi. fertili.	2.807	2.957	2.882	3.15	3.304	3.232	2.856	2.928	2.892	2.061	2.503	2.286
T2- BGA	2.787	2.852	2.823	3.135	3.195	3.165	2.897	2.817	2.857	1.927	2.194	2.061
T3- Azospirillum	2.824	2.792	2.808	3.226	3.208	3.217	2.694	2.705	2.699	2.048	2.167	2.108
T4- Bacillus	2.754	2.824	2.789	3.136	3.229	3.182	2.748	2.791	2.769	1.946	2.038	1.989
T5- Trichoderma	2.749	2.811	2.786	3.077	3.212	3.145	2.629	2.722	2.676	1.964	2.088	2.026
T6- Mycorrhizae	3.073	3.098	3.085	3.167	3.263	3.215	2.774	2.892	2.832	2.216	2.285	2.248
T7- Pseudomonas	2.961	3.123	3.045	3.128	3.295	3.209	2.647	2.788	2.717	2.121	2.183	2.152
T8- T2+ T7	3.067	3.159	3.113	3.244	3.478	3.361	2.782	3.109	2.946	2.304	2.499	2.401
T9- T2+ T6	3.145	3.241	3.193	3.472	3.705	3.588	3.122	3.245	3.183	2.274	2.558	2.416
T10- T3+T4	3.179	3.278	3.228	3.492	3.637	3.564	3.032	3.235	3.134	2.433	2.571	2.501
T11- T3+T4+T7	3.226	3.324	3.275	3.521	3.693	3.607	3.138	3.271	3.205	2.594	2.624	2.609
SE m±	0.038	0.032	0.035	0.054	0.055	0.055	0.123	0.061	0.092	0.075	0.046	0.058
CD at 0.05 %	0.109	0.093	0.101	0.155	0.156	0.155	0.349	0.174	0.261	0.199	0.138	0.165
C.V.%	0.191	0.158	0.175	0.247	0.239	0.243	0.637	0.306	0.472	0.483	0.294	0.389

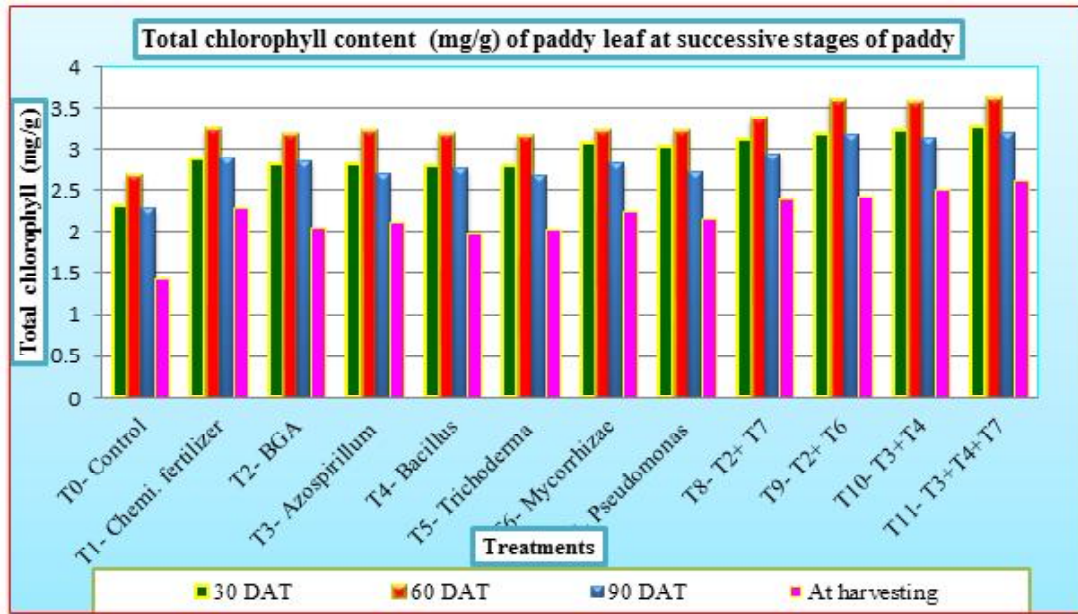


Fig. 4. Effect of biofertilizers on the Total chlorophyll content (mg/g) in the fresh leaves of Paddy (*Oryza sativa* L.) variety, Jaya at various stages of its growth by RBD method

Table-6. Total carbohydrate content (mg/g) in Paddy (*Oryza sativa* L. var. jaya) at successive growth stages by RBD method

Treatments	Total carbohydrate contents (mg/g) FW													
	30 DAT		PD	60 DAT			PD	90 DAT			PD	At harvesting		PD
	2014	2015		2014	2015	2014		2015	2014	2015		2014	2015	
T0- Control	49.174	58.334	53.754	85.463	94.489	89.975	101.63	114.41	108.025	62.461	64.236	63.348		
T1- Chemi. fertili.	63.351	71.469	67.414	114.34	122.91	118.62	130.57	145.66	138.11	66.971	76.693	71.832		
T2- BGA	61.368	68.538	64.953	104.32	121.29	112.81	125.35	140.69	133.02	65.167	72.511	68.839		
T3- <i>Azospirillum</i>	61.836	68.067	64.952	92.817	105.92	99.374	123.04	138.91	130.97	67.461	72.488	69.974		
T4- <i>Bacillus</i>	61.517	67.977	64.744	107.15	103.96	105.55	119.21	130.98	125.11	64.484	71.927	68.205		
T5- <i>Trichoderma</i>	61.698	64.635	63.164	93.538	117.51	105.52	122.06	127.26	124.66	64.551	71.037	67.794		
T6- Mycorrhizae	58.516	70.721	64.618	98.618	122.24	110.43	129.29	138.33	133.81	68.063	78.672	73.367		
T7- <i>Pseudomonas</i>	63.652	68.567	66.109	102.77	122.37	112.57	127.99	145.87	136.93	64.329	77.401	70.865		
T8- T2+ T7	63.057	68.958	66.008	98.698	122.76	110.73	135.38	150.99	143.19	68.958	80.045	74.499		
T9- T2+ T6	66.849	72.149	69.499	116.91	129.07	122.99	139.8	157.98	148.93	72.149	82.249	77.199		
T10- T3+T4	67.887	74.608	71.245	109.91	126.62	118.26	139.55	154.18	146.86	74.608	80.783	77.695		
T11- T3+T4+T7	68.399	79.347	73.873	119.07	129.76	124.42	141.94	157.52	149.73	79.347	83.939	81.643		
SEm±	1.129	1.108	1.118	0.979	1.335	1.157	2.292	1.904	2.098	1.398	1.213	1.305		
CD at 0.05 %	3.193	3.134	3.164	2.771	3.775	3.273	6.483	5.386	5.935	3.954	3.4322	3.693		
C.V.%	0.261	0.2302	0.246	0.136	0.162	0.149	0.258	0.193	0.226	0.295	0.231	0.263		

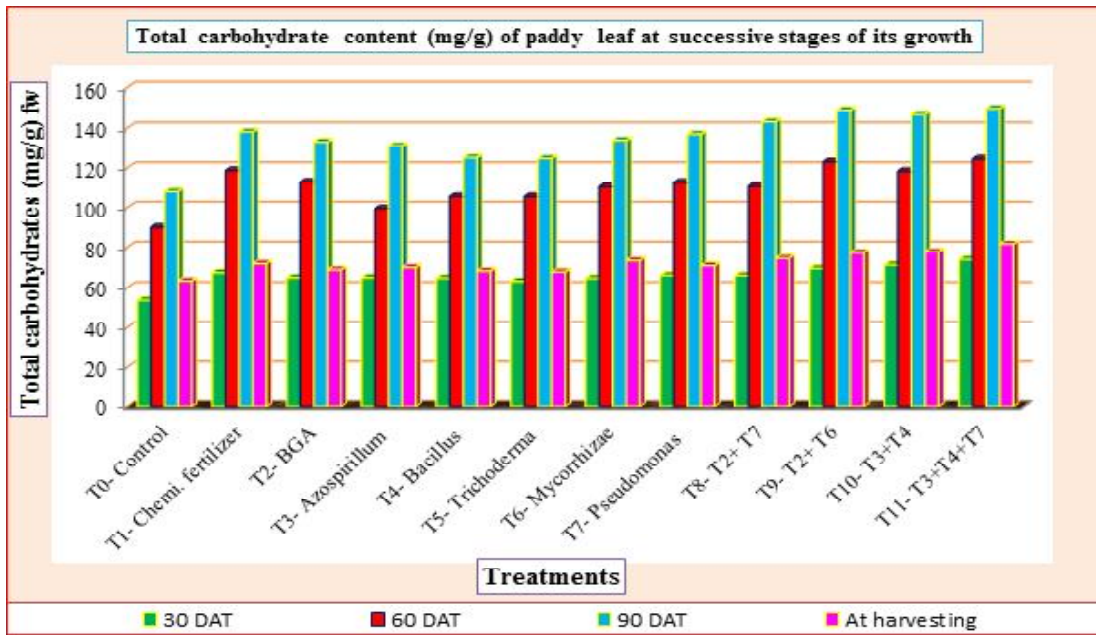


Fig. 5. Effect of biofertilizers on the Total carbohydrate content(mg/g) in the fresh leaves of Paddy (*Oryza sativa* L.) variety, Jaya at various stages of its growth by RBD method

Table-7. Crude protein content(mg/g) in Paddy (*Oryza sativa* L. var. *jaya*) at successive growth stages by RBD method

Treatments	Crude proteins (mg/g DW)											
	30 DAT		PD	60 DAT		PD	90 DAT		PD	At harvesting		PD
	2014	2015		2014	2015		2014	2015		2014	2015	
T0- Control	74.414	89.904	82.159	67.334	87.346	77.342	74.414	65.866	70.146	39.686	42.205	40.945
T1-Chemi.fertili.	94.644	119.69	107.16	80.761	110.13	95.447	94.645	91.848	93.246	46.469	69.059	57.764
T2- BGA	86.651	105.11	95.884	76.913	107.15	92.036	86.651	82.184	84.421	43.236	59.221	51.229
T3- <i>Azospirillum</i>	86.433	101.66	94.049	83.062	102.97	93.018	86.433	84.589	85.511	41.557	58.488	50.019
T4- <i>Bacillus</i>	87.564	111.36	99.464	87.504	100.85	94.178	87.564	80.265	83.914	43.633	57.596	50.614
T5- <i>Trichoderma</i>	81.515	102.45	91.987	75.485	99.979	87.732	81.515	77.787	79.651	42.919	58.984	50.951
T6- Mycorrhizae	89.448	116.99	103.22	76.953	107.93	92.443	89.448	82.883	86.165	41.967	58.647	50.307
T7- <i>Pseudomonas</i>	85.144	125.82	105.48	73.958	103.82	88.893	85.144	84.172	84.658	43.891	64.206	54.045
T8- T2+ T7	93.613	126.93	110.27	73.006	111.91	92.463	93.613	92.225	92.919	48.393	80.881	64.636
T9- T2+ T6	96.707	128.16	112.43	82.586	111.48	97.034	96.707	93.712	95.209	49.206	72.173	60.695
T10- T3+T4	96.961	127.85	112.38	85.283	113.24	99.265	96.965	93.177	95.071	49.928	72.133	61.031
T11- T3+T4+T7	98.512	129.69	114.11	84.886	113.1	98.998	98.512	94.862	96.687	50.872	81.495	66.183
SE m ±	0.023	0.072	0.048	0.032	0.032	0.032	0.446	0.379	0.413	0.188	0.332	0.261
CD at 0.05 %	0.066	0.206	0.136	0.091	0.091	0.091	1.262	1.074	1.168	0.532	0.941	0.736
C.V.%	0.225	0.541	0.383	0.331	0.263	0.297	0.675	0.382	0.528	0.357	0.442	0.412

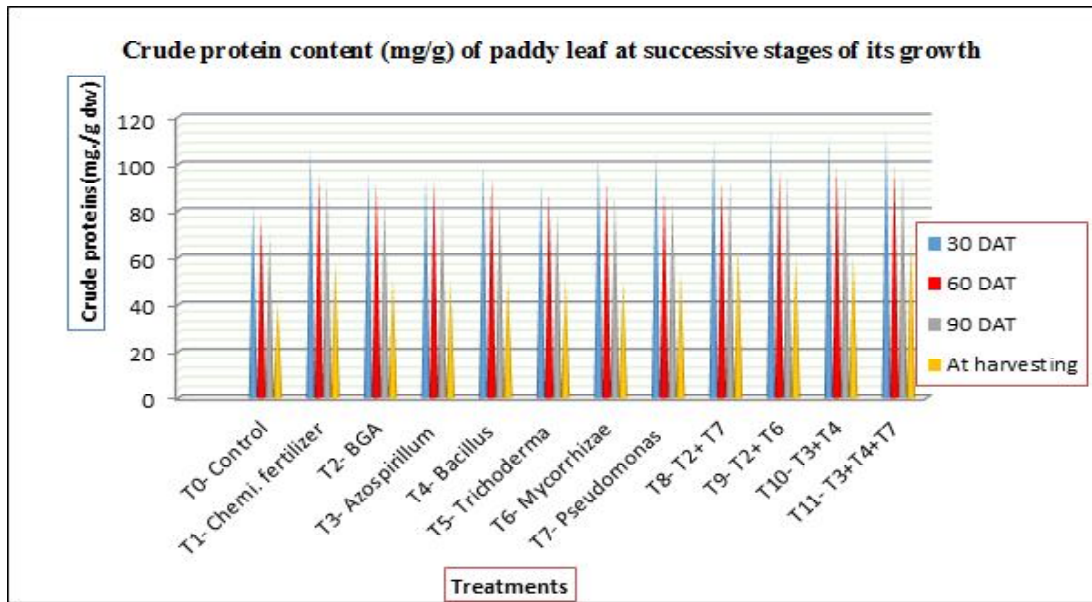


Fig. 6. Effect of biofertilizers on the crude protein content(mg/g) in the fresh leaves of Paddy (*Oryza sativa* L.) variety, Jaya at various stages of its growth by RBD method

Table-8. Proline content(mg/g) in Paddy (*Oryza sativa* L. var. *jaya*) at successive growth stages by RBD method

Treatments	Proline contents (mg/gm. fw.)													
	30 DAT		PD	60 DAT			PD	90 DAT			PD	At harvesting		PD
	2014	2015		2014	2015	2014		2015	2014	2015		2014	2015	
T0- Control	6.672	7.096	6.881	7.196	11.075	9.133	11.316	14.682	12.998	12.506	15.117	13.808		
T1- Chemi. fertili.	7.813	11.606	9.708	9.923	15.436	12.681	13.573	18.514	16.041	15.906	20.116	18.011		
T2- BGA	7.266	9.953	8.614	8.934	13.813	11.371	12.926	18.017	15.468	14.563	17.666	16.115		
T3- Azospirillum	6.983	9.833	8.406	9.236	14.216	11.726	13.961	17.306	15.633	14.525	17.086	15.806		
T4- Bacillus	7.331	9.681	8.506	9.796	13.492	11.643	14.706	16.952	15.828	14.716	18.716	16.716		
T5- Trichoderma	7.213	9.913	8.563	9.951	13.073	11.511	12.686	16.803	14.745	13.711	17.225	15.461		
T6- Mycorrhizae	7.053	9.856	8.455	9.416	13.937	11.673	12.933	18.142	15.536	15.033	19.663	17.348		
T7- Pseudomonas	7.376	10.792	9.083	9.483	14.146	11.813	12.432	17.453	14.943	14.313	21.146	17.728		
T8- T2+ T7	8.133	13.595	10.863	9.866	15.512	12.683	12.271	18.814	15.547	15.733	21.332	18.533		
T9- T2+ T6	8.275	12.139	10.221	10.343	15.752	13.046	13.881	18.736	16.308	16.253	21.542	18.896		
T10- T3+T4	8.391	12.123	10.257	10.053	15.662	12.856	14.332	19.033	16.683	16.296	21.483	18.886		
T11- T3+T4+T7	8.551	13.696	11.123	10.226	15.943	13.085	14.266	19.012	16.638	16.556	21.796	19.172		
SEm±	0.184	0.332	0.265	0.446	0.379	0.413	0.032	0.032	0.032	0.023	0.072	0.048		
CD at 0.05 %	0.532	0.941	0.736	1.262	1.074	1.1681	0.091	0.091	0.091	0.062	0.206	0.136		
C.V.%	0.357	0.442	0.424	0.675	0.382	0.528	0.331	0.263	0.297	0.225	0.541	0.383		

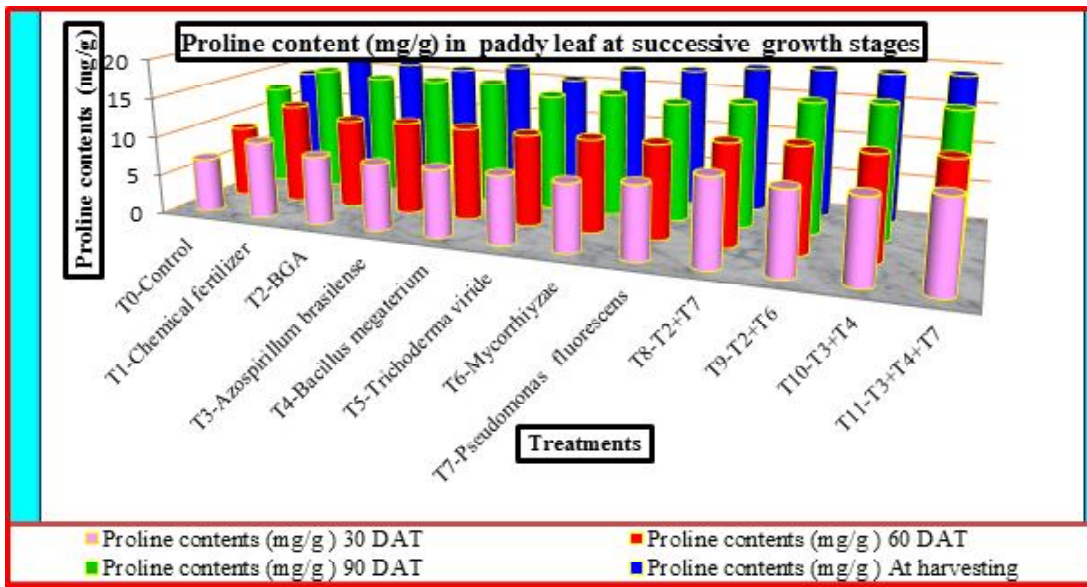


Fig. 7. Effect of biofertilizers on the proline content(mg/g) in the fresh leaves of Paddy (*Oryza sativa* L.) variety, Jaya at various stages of its growth by RBD method

Table-9. Polyphenol content (mg/g) in Paddy (*Oryza sativa* L. var. jaya) at successive growth stages by RBD method

Treatments	Proline contents (mg/gm. fw.)											
	30 DAT			60 DAT			90 DAT			At harvesting		
	2014	2015	PD	2014	2015	PD	2014	2015	PD	2014	2015	PD
T0- Control	1.053	0.753	0.903	4.138	7.423	5.779	9.393	14.309	11.851	16.336	16.326	16.331
T1- Chemi. fertili.	1.107	0.783	0.945	4.785	9.013	6.896	11.373	16.113	13.743	18.693	19.335	19.013
T2- BGA	1.108	0.771	0.939	4.621	8.226	6.423	10.882	17.338	14.107	18.516	18.776	18.646
T3- <i>Azospirillum</i>	1.232	0.806	1.018	5.004	8.651	6.827	10.878	16.043	13.464	18.603	18.503	18.553
T4- <i>Bacillus</i>	1.104	0.756	0.935	4.994	8.533	6.761	10.395	16.684	13.536	18.663	18.023	18.345
T5- <i>Trichoderma</i>	1.117	0.758	0.937	4.68	9.077	6.875	10.557	17.464	14.006	17.852	19.056	18.453
T6- Mycorrhizae	1.245	0.788	1.016	4.976	9.083	7.034	11.348	16.934	14.141	18.814	19.816	19.311
T7- <i>Pseudomonas</i>	1.113	0.789	0.951	4.916	8.623	6.771	11.172	16.436	13.804	18.036	20.145	19.088
T8- T2+ T7	1.219	0.811	1.015	5.115	9.285	7.197	11.864	17.311	14.594	20.087	20.674	20.378
T9- T2+ T6	1.302	0.916	1.109	5.303	9.376	7.346	12.063	18.146	15.105	20.473	20.911	20.691
T10- T3+T4	1.326	0.875	1.098	5.383	9.354	7.366	12.507	18.672	15.588	21.821	21.536	21.678
T11- T3+T4+T7	1.348	0.937	1.142	5.361	9.423	7.392	12.633	19.185	15.907	22.083	21.783	21.933
SE m±	0.012	0.025	0.016	0.051	0.094	0.078	0.332	0.502	0.417	0.393	0.524	0.458
CD at 0.05 %	0.036	0.058	0.047	0.146	0.254	0.232	0.941	1.421	1.181	1.116	1.482	1.297
C.V.%	0.155	0.379	0.263	0.151	0.147	0.149	0.427	0.425	0.426	0.292	0.386	0.339

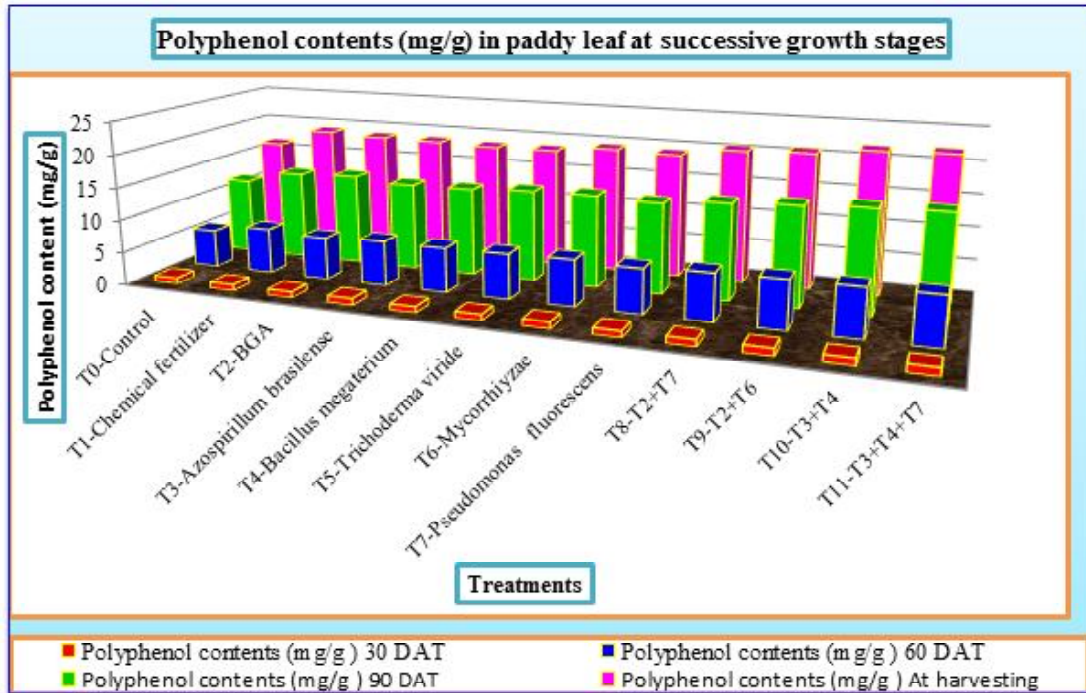


Fig. 8. Effect of biofertilizers on the polyphenol content (mg/g) in the fresh leaves of Paddy (*Oryza sativa* L.) variety, Jaya at various stages of its growth by RBD method

Results on Randomized Block Design (RBD) method conducted during pre-harvest & post-harvest on leaves of paddy for physiological parameters in leaves (photosynthetic pigments, carbohydrates, crude protein, proline and polyphenols) reveal that, with few exceptions, highest values for all parameters were reported for combined application of biofertilizers (*A. brasilense*+*B. megaterium*+*P. fluorescens*). Findings of the study indicate that, for enhanced agricultural production of common rice (*Oryza sativa* L.), combined treatment of biofertilizers, especially of *Azospirillum-brasilense*, *Bacillus megaterium* and *Pseudomonas fluorescens* found to be more effective than the single or dual biofertilizer application. The results of the present study

will be helpful for assessment of effect of different biofertilizers on *Oryza sativa* L. for monitoring and assessment of various parameters in near future. Therefore, data presented in the thesis can be taken as a baseline data for management of the effect of different biofertilizers on growth and yield of common rice. Further, biofertilizers can be used as a cost effective and environment-friendly substitute of the chemical fertilizers. The study recommends organization of programmes to create awareness among farmers for application of combination of biofertilizers in intensive agricultural practices for sustainable development and higher yield.

Encouragement and support provided by Prin. Dr. Ganesh A. Thakur, Principal, Mahatma Phule Arts, Science and Commerce College, Panvel, Dist.–Raigad, Navi Mumbai-410 206 is gratefully acknowledged. Authors are thankful to Prin. Dr. Suryakant Lasune, K. V. P. College, Dombivli for providing laboratory facilities for the present work.

References :

1. Arnon, D. I., (1949). *Archives of Biochemistry* 23: 141.
2. Aswathy, J. C., P. Shalini, Pillai., Jacob, John., and K. S. Meenakumari. (2020). *Journal of Pharmacognosy and Phytochemistry*, 9(5): 2920-2923.
3. Bates, L. S., R. P. Waldren, and I. D. Tare, (1973). *Plant Soil*, 39: 205–207.
4. Bouman, B.A.M., and T.P. Tuong (2000). *Agric. Water Manag.* 1615: 1–20.
5. Carrijo, D. R., M. E. Lundy, and B. A. Linquist, (2017). *Field Crops Res.*, 203: 173–180.
6. Chandrasekaran, B., K. Annadurai, and R. Kavimani, (2007) A text book of Rice Science. Scientific Publishers (India). Jodhpur, 8.
7. Chunthaburee, Sumitahnun., Anoma, Dongsansuk., Jirawat, Sanitchon., Wattana, Pattanagul., and Piyada, Theerakulpisut. (2016). *Saudi Journal of Biological Sciences*, 23: 467–477. <http://dx.doi.org/10.1016/j.sjbs.2015.05.013>.
8. Doni, F., N. S. M. Suhaimi, M. S. Mispan, F. Fathurrahman, B. M. Marzuki, J. Kusmoro, and N. Uphoff, (2022). *Int. J. Mol. Sci.*, 23: 737. <https://doi.org/10.3390/ijms23020737>.
9. Doni, Febri., Anizan, Isahak., Che, Radziah Che Mohd Zain., and Wan, Mohtar Wan Yusoff., (2014). Physiological and growth response of rice plants (*Oryza sativa* L.) to Trichoderma spp. inoculants. *AMB Express* 4: 45 <http://www.amb-express.com/content/4/1/45>.
10. Fernandes, Peter. and Satish, A. Bhalerao (2015). *Int. J. Adv. Res. Biol. Sci.*, 2(4): 127-130.
11. Folin, O., and W. Denis, (1915). *The Journal of Biological Chemistry*, 22(2): 305-308.
12. Ghimire, A. R., A. Nainawasti, T. B. Shah, and S. Dhakal, (2021). *SAARC J. Agric.*, 19(1): 57-69. DOI: <https://doi.org/10.3329/sja.v19i1.54778>.
13. Gimhavanekar, V. J., M. M. Burondkar., V. V. Dalvi., S. G. Bhave., S. S. Desai., S. G. Mahadik., S. S. Chavan., and A. K. Shinde (2020). *Journal of Pharmacognosy and Phytochemistry*, 9(5): 1616-1624.
14. Guimaraes, Cleber Morais., Luis, Fernando Stone., Adriano, Pereira de Castro., and Odilon, Peixoto de Morais Júnior., (2015). *Pesq. agropec. bras., Brasília*, 50(7): 534-540, jul. 2015. DOI: 10.1590/S0100-204X2015000700003.
15. Hossain, M. F., B. C. Sarker., M. Kamaruzzaman., M. A. Halim., and M. M. Ahmmed (2015). *Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, 8(6): Ver. II, PP. 61-67. DOI: 10.9790/2380-08626167.
16. Ichsan, Cut Nur., Bakhtiar., Efendi., Sabaruddin., (2020). Morphological and physiological change of rice (*Oryza sativa* L.) under water stress at early season. ICATES 2020. IOP Conf. Series: Earth and Environmental Science 644 (2021) 012030. IOP Publishing. doi:10.1088/1755-1315/644/1/012030.

17. Khan, Haider Iqbal. (2018). *Rice Science*, 25(6): 357-362. <http://dx.doi.org/10.1016/j.rsci.2018.10.006>.
18. Kumar, J., A. Ramlal, D. Mallick, and V. Mishra, (2021). An Overview of Some Biopesticides and Their Importance in Plant Protection for Commercial Acceptance. *Plants*, 10, 1185. <https://doi.org/10.3390/plants10061185>.
19. Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R. J. Randall, (1951). *J. Biol. Chem.*, 193: 265 (The original method).
20. Majeed, A., Z. Muhammad, S. Islam, Z. Ullah and R. Ullah (2017). *PSM Microbiol.*, 2(2): 47-50.
21. Malo, M., A. Ghosh, D. Dutta, and K. Murmu (2018). *Journal of Pharmacognosy and Phytochemistry*, 7(1): 576-580.
22. Mhalevilie, Thorie., N. C. Sarkar, Atou Kharutso, (2013). *International Journal of Bio-resource and Stress Management*, 4(3): 400-403.
23. Mishra, Soumya., Manoranjan, Kar., S. P. Monalisa., and Alaka, Sahu., (2017). *Int. J. Curr. Microbiol. App. Sci.*, 6(10): 157-165. doi: <https://doi.org/10.20546/ijcmas.2017.610.020>.
24. Mudoi, Tiluttama., and Priyanka, Das., (2020). *Int. J. Curr. Microbiol. App. Sci.*, 9(05): 402-414. doi: <https://doi.org/10.20546/ijcmas.2020.905.045>.
25. Nadim, M. K. A., M. Mitu., M. M. Islam., M. S. Haque., S.M. A. Alim., S. E. Akter., M. G. Mortuza. and K. U. Ahmed. (2022). Biochemical and Molecular Characterization of Some Advanced Mutant Rice (*Oryza sativa* L.) Genotypes. *SAARC J. Agric.*, 20(1): 41-53. DOI: <https://doi.org/10.3329/sja.v20i1.60612>.
26. Ojha, Surendra Kumar., Jane C Benjamin, and Ajay Kumar Singh, (2018). *Journal of Pharmacognosy and Phytochemistry*, 7(4): 830-832.
27. Panse, V. G., and P. V. Sukhatme., (1967). "Statistical Methods for Agricultural Workers," 2nd Edition, Indian Council of Agricultural Research, New Delhi.
28. Rakesh Kumar, Narendra Kumawat, and Yogesh Kumar Sahu., (2017). *Popular Kheti*, 5(4): 63-66.
29. Rana, R., S. Ramesh and P. Kapoor (2013). *Popular kheti* 1(1): 2321-0001.
30. Sadasivam S. and A. Manickam, (2004) "Biochemical Methods," 2nd Edition, New Age International (P) Limited Publishers, New Delhi.
31. Sahoo, R., D. Bhardwa, and N. Tuteja, (2013). Biofertilizers: A sustainable eco-friendly agricultural approach to crop improvement. (ed. S.G. tuteja) *Plant Acclimation to Environmental Stress*. Springer. pp. 403-432.
32. Sarker, U.K., M.R. Uddin, M.A.R. Sarkar, M.A. Salam, and A.K. Hasan, (2017). *Archives of Agriculture and Environmental Science*, 2(4): 247-256, DOI: 10.26832/24566632.2017.020401.
33. Selvakumar, S., S. Sakthivel., Akihiko, Kamoshita., R. Babu., S. Thiyageshwari., and A. Raviraj. (2020). *Current Journal of Applied Science and Technology*, 39(20): 123-131. Article no.CJAST.59041.
34. Singh, Rama Kant, Pankaj Kumar, Birendra Prasad, and S. B. Singh, (2015). *Internat. Res. J. Agric. Eco. & Stat.*, 6(2): 386-391.
35. Singh, S., R. N. Singh, J. Prasad, and B. Kumar, (2002). *J Ind Soc Soil Sci*, 50(3): 313-314.
36. Sureshkumar, R., and B. J. Pandian., (2017). *Chem Sci Rev Lett.*, 6(22): 1321-

- 1326.
37. Take-Tsaba, A. I., A. S. Juraimi, M. R. Yusop, R. Othman, and A. Singh, (2018). *The J. Anim. Plant Sci.*, 28(6): 1774-1786.
38. Thakur, A.K., Norman, Uphoff. and Edna, Antony., (2010). *Expl Agric.*, 46(1): 77–98. Cambridge University Press 2009, doi:10.1017/S0014479709990548.
39. Timung, Bikha., B. Bharali., and Milon, Jyoti Konwar., (2017). *Journal of Pharmacognosy and Phytochemistry*, 6(6): 1636-1640.
40. Wencheng Ding, Xinpeng Xua, Ping Hea, Sami Ullaha, Jiajia Zhanga, Zhenling Cuib, Wei Zhoua, (2018). *Field Crops Research*, 227:11–18. <https://doi.org/10.1016/j.fcr.2018.08.001>.
41. Xin, W., L. Zhang, W. Zhang, J. Gao, J. Yi, X. Zhen, M. Du, Y. Zhao, and L. Chen, (2022). *Agronomy*, 12: 358. <https://doi.org/10.3390/agronomy12020358>.