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Impact of biozyme on physiological and biochemical for optimization of rice (*Oryza sativa* L.) yield

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Abstract
Biozyme is a biodegradable and non-toxic for the plant and soil health containing growth hormones, elements, minerals and vitamins and highly compatible with fertilizers and pesticides, it also increases the resistance of plants against various pests, diseases and climatic stress. Keeping in view of its importance a field trial was formulated and conducted at Research cum Industrial Farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during *kharif* 2018-19. The Indira Rajeshwari (IGKV- R1) rice genotype was collected from IGKV, Raipur and seven treatments were consisted *i.e.* T_1 No treatment, RDF only 120: 60: 40 (control), T_2 RDF+ biozyme granule@15 kg/ha at 15 DAT, T_3 RDF+ biozyme grain @ 15 kg/ha at 15DAT and tillering stage, T_4 RDF + Biozyme grain@ 15 kg/ha at 15 DAT+ Biozyme liquid @ 500 ml/ha and 500ml/ha at boot leaf stage, T_5 RDF+ Biozyme granule @ 15 kg /ha at 15DAT and tillering stage+ 500 ml/ha at boot leaf stage, T_6 RDF+ Biozyme granule at 15DAT + Amaze X Gr.@ 10 kg/ha at tillering stage and T_7 RDF+ Biozyme granule at 15DAT + Amaze X Gr.@ 10 kg/ha @ 500 ml /ha at tillering stage+ Biozyme liquid at boot leaf stage in Randomized Block Design (RBD) with three replications. The biozyme was applied in form granular, liquid alongwith recommended dose fertilizer and the liquid treatments were through garden spray bottle plastic nozzle hand pressure spray head whereas; the granular were done by broadcasting method.
The physiological, biochemical and yield traits were recorded under the application of (T_4 -RDF+ biozyme grain @ 15 kg/ha at 15DAT and tillering stage) as compared to control (T_1 RDF only) might be due to biozyme regulated plant bio-physiological activities which in turn resulted in higher chlorophyll content in leaves that's help in maintaining higher photosynthetic activity.

Keywords: Rice (*Oryza sativa* L.), biozyme, rice physiology, zymes, seaweed extract

Introduction
Rice is a cereal crop that provides the staple food for more than half of the world population and is grown in over hundred countries. It is the main food crop in the world and nearly 40% of thei world population consumes rice as the major staple food. In India, it occupies an area of 43.86 million ha with total production of 105.48 million tons. In *kharif* season, rice production was 91.39 million tons. Chhattisgarh popularly known as 'Rice bowl of India' occupies an area around 3.61 million hectares with the production of 5.48 million tones and productivity 1517 kg ha^{-1} (Anonymous, 2013 b) [1]. The biozyme is an eco-friendly non-toxic commercial growth stimulants which influences the plant physiological systems at low concentrations and known to be rice in cytokinin and auxin precursor, enzyme and hydrolyzed protein is a store house of naturally occurring nutrients derived from *Ascophyllum nodusom*. Biozyme is extremely compatible with pesticides and fertilizers rises the resistance of crop plants against various disease, pests and climatic stress. These zymes are present in solid and liquid form and are being used as either spraying or top dressing on the crop canopy at key stages of crop growth. It is beneficial in vegetative part as well as in grain production (Kumar *et al.* 2016) [3]. The biostimulents fertilizer are chosen not only due to their essential nutrient (NPK) content but also because of the presence of metabolites similar to plant growth regulators. Seaweed extract as liquid fertilizer (SLF) have introduced in the market for the simple reason that they content many growth promoting hormones like auxin, gibbrellins, trace elements, vitamins, amino acidsi and micronutrients. The growth promoting effects of seaweed extracts on seed germination, vegetative growth and biochemical characteristics in agriculture crop.The seaweed liquid extract (SLE) exhibited promoting outcome on growth and yield parameters at low concentration (1.5%). The variance effect in the content of chlorophyll pigments, protein vitamin- C, non reducing sugar, reducing sugar and the activity of nitrate reductase in the leaves of SLE tested seedlings. An addition of inorganic fertilizer with biozyme could improve the soil physico-chemical properties and thus help in better soil environment suitable for increased crop growth and yield (Mancuso *et al.*, 2006) [4] Resistant.

Materials and Methods

The field experiment was carried out at Research cum instructional farm and the laboratory analysis was done in the Department of Plant Physiology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, and Krishak Nagar Raipur (C.G.) during *Kharif* 2018-2019.

The Indira Rajeshwari (IGKV- R1) rice genotype was collected from IGKV, Raipur and seven treatments were consisted *i.e.* T_1 No treatment, RDF only 120: 60 : 40 (control), T_2 RDF+ biozyme granule@15 kg/ha at 15 DAT, T_3 RDF+ biozyme grain @ 15 kg/ha at 15DAT and tillering stage, T_4 RDF + Biozyme grain@ 15 kg/ha at 15 DAT+ Biozyme liquid @ 500 ml/ha and 500ml/ha at boot leaf stage, T_5 RDF+ Biozyme granule @ 15 kg /ha at 15DAT and tillering stage+ 500 ml/ha at boot leaf stage, T_6 RDF+ Biozyme granule at 15DAT + Amaze X Gr.@ 10 kg/ha at tillering stage and T_7 RDF+ Biozyme granule at 15DAT + Amaze X Gr.@ 10 kg/ha @ 500 ml /ha at tillering stage+ Biozyme liquid at boot leaf stage in Randomized Block Design (RBD) with three replications. The biozyme was applied in form granular, liquid alongwith recommended dose fertilizer and the liquid treatments were through garden spray bottle plastic nozzle hand pressure spray head whereas; the granular were done by broadcasting method.

A. Physiological Parameters

(i)Total dry matter production (g)

The rice plants of each genotype were uprooted at tillering, flowering and maturity stage. Each plant parts were separated *viz.* stem, panicle, leaves and air dried over night at room temperature. The plant parts were dried in a hot air oven maintained at 80^0C for 48 hours to obtain constant dry weight followed by measurement of dry weight. The sum of the mean dry weight of all the plant parts has taken separately. The dry weight of different plant parts and total dry weight was recorded.

(ii) Leaf area index (LAI)

The five plant samples were taken from the experimental plot at tillering and at 50% flowering stage and the mother tillers were separated and all the leaves were removed and kept into a beaker to avoid the leaf rolling. The maximum length and width of leaves were measured and was kept till constant weight. Similarly leaves from rest of the tillers were removed and was kept for drying up to constant weight. For leaf area, a factor 0.75 (at panicle and flowering) and 0.67 (at maturity) was used. Leaf area index was calculated as follows:

Leaf area = Length x Width x Correction factor (K)

$$\text{Leaf area index(LAI)} = \frac{\text{Leaf area cm}^2}{\text{Ground area cm}^2}$$

(iii) Flag leaf area (cm^2)

The upper most fully expanded leaf of the mother tiller was selected for the estimation of flag leaf area at flowering stage. The maximum length and maximum width of flag leaf were recorded at flowering stage and a factor of 0.75 was used to calculate the flag leaf area. It was expressed as cm^2.

Flag leaf area = Length x Width x k (Factor 0.75)

B. Biochemical parameters

(i) Chlorophyll content a, b and total chlorophyll (mg/g fresh weight)

Procedure

Total chlorophyll content was estimated by the method described Arnon (1949).

1. 200 mg of leaf samples (2^{nd} leaf) were dipped in 10 ml of 80% acetone in a graduated glass tube.
2. The glass tubes were incubated in dark at 4^0C for 48 hrs.
3. After 48 hrs absorbance were taken at 645 nm and 663 nm.
4. Quantification of the total chlorophyll, chlorophyll a and chlorophyll b content in an 80% acetone extract was done by following equation:

Total chlorophyll (mg/g fresh weight) =$20.2(A_{645})$-$8.02(A_{663})$ x dilution factor

Chlorophyll a (mg/g fresh weight) = $12.7(A_{663})$-$2.69(A_{645})$ x dilution factor

Chlorophyll b (mg/g fresh weight) = $22.9\ (A_{645})$ - $4.68\ (A_{663})$ x dilution factor

Where, A_{663} is the absorbance at 663 nm and A_{645} is the absorption at 645.

(ii) Amylose content (%)

Procedure

Reagent:

1. Iodine reagent (1g Iodine + 10g KI in water, makeup volume 500ml)
2. 1N Acetic acid

Clean rice sample were kept in the room at least two days to develop uniform moisture content and grinding of the samples done by pestle and mortar.100 mg of dried rice powder was put in a conical flask. 1 ml of 95% ethanol and 9 ml of 1 M NaOH added. The suspension was put in vigorously boiling water bath for 10 min. cool at lab ambient temp for 10 min then DW was added to make 100 ml solution. 5 ml of aliquot of the solution was transferred to 100 ml VF. Adjust Ph, 1 ml of 1 N acetic acid was added. Added 0.2% Iodine solution and the volume was made up to 100 ml with distilled water this was kept for 20 minutes afterwards transmission at 620nm was read with the use of blank. The percent amylase was calculated with the use of standard curve of potato amylase of the concentration 400 mg/ml under identical conditions.

C. Yield parameters

(i) Number of filled and unfilled grains/panicle

The total numbers of filled and unfilled grains present in the panicle were counted after harvesting of the crop.

(ii) Sterility percentage (%)

Total number of unfilled grains of ten hills was counted amongst the total grains each plot and sterility percentage was worked out with the help of the following formula:-

$$\text{Sterility percentage} = \frac{\text{number of unfilled grain}}{\text{total number of grain}} \text{X}100$$

(iii) Grain yield (kg /ha)

The weight of grains of each individual plot was recorded in kg/ha.

Results and Discussion

A. Physiological Parameters

- **Total dry matter production (g) Leaf area index (LAI) and Flag leaf area (cm^2)**

The influence of biozymes on total dry matter production (TDM) (g/m^2) at tillering, flowering and maturity stage, leaf area index and flag leaf area (cm^2) at flowering stage in rice genotype are presented in Table 1.

The rice plants increased progressively with the advancement of growth stages and reached rice peak at maturity. The total dry matter significantly varied among the treatments and the maximum was found under T_4-100% RDF + Biozyme granule @ 15kg/ha + Biozyme liquid @ 500 ml/ha (395.3), (572.6), and (1324.6) whereas, minimum was recorded under T_1 (RDF only) (275.0), (441.6) and (794.3) at tillering, flowering and maturity stage respectively. It might be due to the favorable effects of seaweed extracts to supply of growth promoting hormones, several nutrients and other bio-stimulating substances in readily available form. Pramanik *et al.* (2013) also found that the foliar application of seaweed extract on green gram increased dry matter accumulation with RDF over control

The leaf area index (LAI) is the most important parameter of growth analysis. It is a measure of canopy coverage per unit area and directly involved in photosynthesis. The maximum LAI was recorded in T_4-100% RDF + Biozyme granule @ 15kg/ha + Biozyme liquid @ 500 ml/ha (6.04) and minimum was recorded in control (T_1- RDF only) (3.65) at flowering stage respectively. The increasing trend of LAI in treated can be attributed to the positive effect of biozyme on both leaf development and leaf area duration of the crop variety. Nayak *et al.* (2020) also reported that the progressive increment in LAI of the variety up to certain days may be due to the fact that addition of nutrient triggers increased number of leaves per plant and expansion of individual leaf.

The flag leaf area was significantly maximum recorded under 100% RDF + Biozyme granule @ 15kg/ha + Biozyme liquid @ 500 ml/ha (T_4) and minimum was recorded in control plot (T_1) (41.9) and (33.3) at flowering stage respectively. Similar findings were also reported Nayak *et al.* (2020) [5].

B. Biochemical parameters

- **Chlorophyll content a, b and total chlorophyll (mg/g fresh weight) and Amylose content (%)**

The influence of different formulation of biozyme on chlorophyll a, b and total chlorophyll (mg/g fresh weight) and Amylose content (%) of rice genotypes at flowering stage are presented in Table. 2.

The total chlorophyll, chlorophyll a and b were significantly affected by different formulation of biozyme application. The total chlorophyll, chlorophyll a and b were significantly higher under application of 100% RDF + Biozyme granule @ 15kg/ha + Biozyme liquid @ 500 ml/ha (T_4) (2.66), followed by 100% RDF + Biozyme granule @15 kg/ha (T_2) (1.78) and 100% RDF + Biozyme granule @15 kg/ha + Biozyme liquid @ 500 ml/ha (T_5) (1.75) whereas, minimum was recorded in control plot (T_1- RDF only) (0.92) as compared to all the treatments. Ten Singh *et al.* (2017) [7] reported that the influence of Seaweed extract of *Rosenvigea intricata* with or without chemical fertilizer on chlorophyll content of *Abelmoschus esculentus* was analyzed and found maximum at 20% SLF with or without chemical fertilizer.

The amylase content was significantly differed in all the tested plots. The maximum amylose content was numerically higher in (T_4) 100% RDF + Biozyme granule @ 15kg/ha + Biozyme liquid @ 500 ml/ha (33.0), followed by T_5 100% RDF + Biozyme granule @15 kg/ha + Biozyme liquid @ 500 ml/ha (T_5) (32.65) and $T_2$100% RDF + Biozyme granule @15 kg/ha (27.52) and minimum was recorded in T_1 RDF only (25.31). It may be due to the biozyme and amaze contains several enzyme and nutrient which helps in increasing starch content. Similar results were also reported by Kumar *et al.* (2000) [2].

C. Yield parameters

- **Number of filled, unfilled grains/panicle, Sterility percentage (%) and Grain yield (kg /ha)**

The influence of different formulation of biozyme on number of filled, unfilled grains/panicle, sterility percentage (%) and Grain yield (kg /ha) of rice genotypes at are depicted in Table. 3 and Fig.1. The number of filled grains/panicle was differed significantly due to biozyme and was recorded maximum in application of (T_4) 100% RDF + Biozyme granule @ 15kg/ha + Biozyme liquid @ 500 ml/ha (139.0) as compared to 100% RDF only (T_1) might be due to better utilization of resources in the plots receiving growth hormones, micronutrients, enzymes, proteins, vitamins etc through the addition of *Ascophyllum nodosum, Laminaria spp and Saccharina latissima* extracts. Similarly observed by Nayal *et. al,* (2020)

The maximum number of unfilled grains/panicle and sterility percentage (%) were recorded in 100% RDF + Biozyme granule @ 15 kg/ha a +Amaze-x granule @10 kg/ha + Biozyme liquid @ 500 ml/ ha (T_7) (13.66) (14.03%) followed by T_1 (12.33) (12.92%) under the application of 100% RDF only whereas minimum unfilled grains/panicle was recorded (T_5) (7.33) under the application of 100% RDF + Biozyme granule @15 kg/ha + Biozyme liquid @ 500 ml/ha and sterility percentage (%) under the application of 100% RDF + Biozyme granule @ 15kg/ha + Biozyme liquid @ 500 ml/ha (T_4) (8.64%) respectively.

The grains yield (kg/ha) was recorded exhibited maximum under (T_4) under the application of 100% RDF+ Biozyme granule @ 15kg/ha + Biozyme liquid @ 500 ml/ha) followed by (T_3 100% RDF +Biozyme Gr. @ 15 kg/ha) as compared to control (T_1 100% RDF only). The grains yield was recorded higher in T_4 (5600 kg/ha), T_3 (5560kg/ha) and T_5 (5556kg/ha) under (T_4 100% RDF+ Biozyme granule @ 15kg/ha + Biozyme liquid @ 500 ml/ha) and (T_3) 100% RDF +Biozyme Gr. @ 15 kg/ha respectively as compared to control plot. However, minimum was recorded in T_1 (4180kg/ha) under the application of 100 % RDF only in tested rice genotype. The increment of grain yield at higher with the application of biozyme granule at suitable rice growth stage might be due to optimum absorption of nutrient and other minerals which increase the production and translocation of the food materials from source to sink. Similar results were also reported by Kumar *et al.* (2016) [3]

Table 1: Influence of biozymes on total dry matter (g/m^2), leaf area index and flag leaf area (cm^2) of rice (*Oryza sativa* L.) genotype

Treatment detail	Total dry matter (g/m^2)			LAI	Flag leaf area (cm^2)
	Tillering	Flowering	Maturity		
T_1- 100% RDF	275.0	441.6	794.3	3.65	33.3
T_2 -100% RDF + Biozyme granule @15 kg/ha at 15DAT	344.2	557.0	1077.6	5.24	35.9
T3 -100% RDF +Biozyme Gr. @ 15 kg/ha at 15DAT and tillering stage	370.33	540.0	1033.3	4.35	38.9
T_4 - 100% RDF + Biozyme granul@15kg/ha at 15DAT + Biozyme liquid @ 500 ml/ha at tillering and boot leaf stage,	395.3	572.6	1324.6	6.04	41.9

T_5 -100% RDF + Biozyme granule @15 kg/ha at 15DAT and tillering stage + Biozyme liquid @ 500 ml/hac at boot leaf stage	388.6	567.0	1264.6	5.23	38.9
T_{-6} 100% RDF+ Amaze-x granule @ 10 kg/ha at tillering stage,	385.0	549.6	1156.3	5.57	38.1
T_7- 100% RDF + Biozyme granule @ 15 kg/ha at 15 DAT+Amaze-x granule @10 kg/ha at tillering stage + Biozyme liquid @ 500 ml/ ha at boot leaf stage	345.0	55.0	1007.0	5.16	38.9
C.D.	34.77	12.08	58.21	1.16	4.62
SE± (m)	11.28	3.92	18.89	0.37	1.49

Table 2: Influence of biozymes on chlorophyll content (Chl a, Chl b and total chl mg/g fresh weight and amylase content (%) of rice (*Oryza sativa* L.) genotype

Treatment detail	Chlorophyll content (mg/g fresh weight)			Amylose content (%)
	Chlorophyll a	Chlorophyll b	Total chlorophyll	
T_1- 100% RDF	0.61	0.92	0.92	25.31
T_2 -100% RDF + Biozyme granule @15 kg/ha at 15DAT	0.78	1.78	1.78	27.52
T_3 -100% RDF +Biozyme Gr. @ 15 kg/ha at 15DAT and tillering stage	0.61	1.33	0.33	28.16
T_4 - 100% RDF + Biozyme granul@15kg/ha at 15DAT + Biozyme liquid @ 500 ml/ha at tillering and boot leaf stage,	0.99	2.66	2.66	35.0
T_5 -100% RDF + Biozyme granule @15 kg/ha at 15DAT and tillering stage + Biozyme liquid @ 500 ml/hac at boot leaf stage	0.68	1.75	1.75	32.65
T_6- 100% RDF+ Amaze-x granule @ 10 kg/ha at tillering stage,	0.80	1.71	1.71	27.26
T_7 - 100% RDF + Biozyme granule @ 15 kg/ha at 15 DAT+Amaze-x granule @10 kg/ha at tillering stage + Biozyme liquid @ 500 ml/ ha at boot leaf stage	0.72	1.38	1.38	26.93
C.D.	0.18	0.90	0.24	4.13
SE± (m)	0.05	0.29	0.07	1.34

Table 3: Influence of biozymes on filled grain, unfilled grain/panicle, sterility percentage (%) and grain yield (kg/ha) of rice (*Oryza sativa* L.) genotype

Treatment detail	Filled grain/ panicle	Unfilled grain/ panicle	Sterility (%)	Grain Yield (kg/ha)
T_1- 100% RDF	100.33	12.33	12.92	4180
T_2 -100% RDF + Biozyme granule @15 kg/ha at 15DAT	105.0	11.33	8.28	4940
T_3 -100% RDF +Biozyme Gr. @ 15 kg/ha at 15DAT and tillering stage	104.33	11.33	9.33	5560
T_4 - 100% RDF + Biozyme granul@15kg/ha at 15DAT + Biozyme liquid @ 500 ml/ha at tillering and boot leaf stage,	136.33	10.33	8.64	5600
T_5 -100% RDF + Biozyme granule @15 kg/ha at 15DAT and tillering stage + Biozyme liquid @ 500 ml/hac at boot leaf stage	116.34	7.33	6.62	5556
T_6- 100% RDF+ Amaze-x granule @ 10 kg/ha at tillering stage,	119.66	9.00	10.35	4444
T_7 - 100% RDF + Biozyme granule @ 15 kg/ha at 15 DAT+Amaze-x granule @10 kg/ha at tillering stage + Biozyme liquid @ 500 ml/ ha at boot leaf stage	113.0	13.66	14.03	4816
C.D.	9.89	3.63	4.87	2.41
SE± (m)	3.21	1.18	1.58	0.78

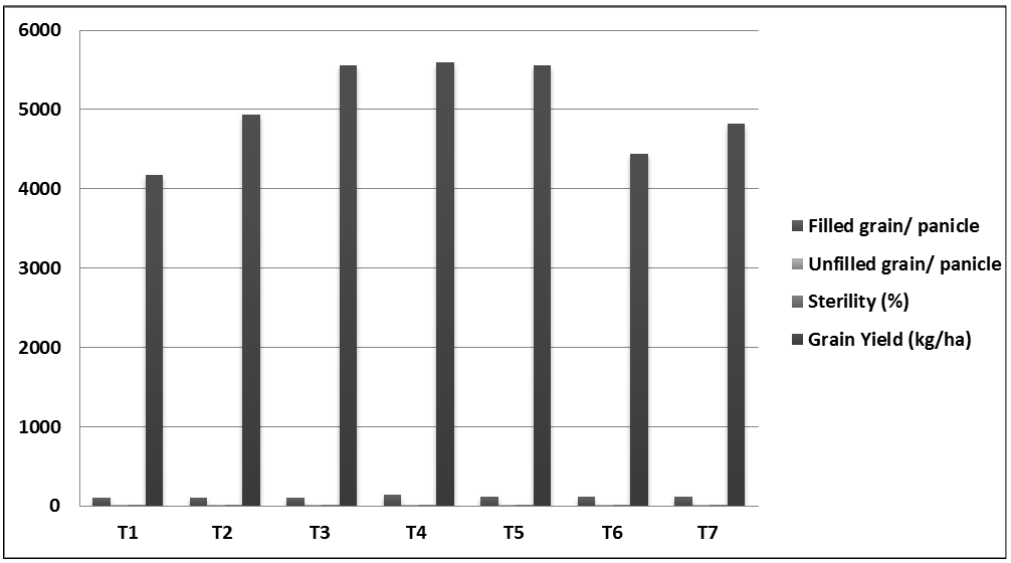

Fig 1: Influence of biozymes on filled grain, unfilled garin/panicle, sterility percentage (%) and grain yield (kg/ha) of rice (*Oryza sativa* L.) genotype

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