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A Novel Approach: Stimulatory Effect of Bioenzyme supplementation on quality of Mung bean

S. Patidar[1], A. Bafna[1], T. Rangwala[1], N. Vyas[2] and R. Gupta[2]

[1]Department of Biochemistry, Govt. Holkar Science College, Indore 452 017, M.P., India
[2]Noble Alchem Pvt. Ltd., Sanwer Road, Indore (M.P.), India

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ABSTRACT

Organic farming nowadays is a major area of interest for research. It is the best way to restore disturbed ecological balance due to excessive use of chemicals in agriculture like fertilizers, pesticides, insecticides, etc. The present study was designed to evaluate the potential of eco- friendly product bioenzyme on biochemical and oxidative stress parameters of mung (*Vigna radiata*). A field experiment was conducted at Loharpipliya village, district Dewas (M.P.) during March-May 2022. Various dilutions of Bioenzyme were sprayed at the interval of 15 days after the two leaves stage of the plant. Four treatments were designed – Control (without any treatment), T1 (1ml bioenzyme/L water), T2 (2 ml bioenzyme/L water), and T3 (3 ml bioenzyme/L water). Photosynthetic pigments were significantly increased in all the treatments involving bioenzyme collated to control. A highly significant increment in carbohydrate and protein content in leaves of mung was reported with T2 treatment. Oxidative stress assessed in terms of MDA and proline was observed to decrease significantly with all treatments. Among all the three concentrations of bioenzyme used in the present study, the best results were obtained with the spraying of 2ml bioenzyme/l water (T2). Bioenzyme can be a good alternative for farmers to improve the quality of mung without the use of harmful chemicals to avoid adverse effects on soil and crop quality. Though further research is required for use of bioenzyme with other crops too.

Key words: *Carbohydrate, MDA, Proline, Protein, Vigna radiata.*

Introduction

Agriculture is one of the most significant economic activities across all parts of the world. India has the second largest area of arable land in the world and is a major producer of a number of agricultural products. Farmers are facing many problems due to the volatile climate which results in poor quality and reduced yield of the crops. Although chemical fertilizers and supplements used to enhance production accomplish the plants or crop needs but they have drawbacks like adversely affects the pH of soil and ground water, kills eco-friendly microflora and also very harmful for human health etc. The above problem can be resolved by use of eco-friendly nontoxic soil supplement and growth stimulant "Bioenzyme".

In many countries kitchen waste is currently landfilled together with other municipal wastes (FAO). Due to its nutrient rich composition, can be utilized as an enzyme resource. Such conversion of Kitchen waste is potentially more profitable for Indian Farmers. Bioenzyme is also known as garbage enzyme which was invented byDr. Rosukon in Thailand from residues such as vegetables, fruits or its peels which are abundantly available in supermarket of Malaysia (Lim *et al.*, 2016).

Bioenzyme is known fertilizer having essential

nutrient (NPK) content as well as metabolites similar to plant growth regulators. Bioenzyme influences the plant physiological systems at low concentration. It can act as natural pesticide, insecticide, herbicide and fertilizer, induce the uptake of nutrients in seedlings of chick pea (Moloto *et al.*, 2018).

Application of bioenzyme accelerate activity of enzymes involved in physiological processes which in turn hydrolyse starch and helps in the metabolic activity during the change of available starch into sugar content (Jain, 2006). It contains nutrients in a naturally chelated form which helps to improve cell division and cell enlargement resulting into better chlorophyll content and increased production. (Manna *et al.*, 2015).

Mung bean (*Vigna radiata)* is a third economically most important crop in India grown in nearly 16% of the total pulse area. It has great value as food and is a cheap source of protein for direct human consumption (Mubarak, 2005). Mung bean is not only rich in protein but also enrich content of nitrogen in soil.

Materials and Methods

Mung bean (*Vigna radiata*) seeds MI731-3 variety was purchased from Indian Agriculture Research Institute (IARI) Indore, Madhya Pradesh.

Three different concentrations of bioenzyme were supplied at the interval of every fifteen days after sowing.

C- Without any treatment

T1 – (1 ml bioenzyme/L water)

T2- (2 ml bioenzyme/L water)

T3- (3 ml bioenzyme/L water)

Photosynthetic pigments, biochemical and oxidative stress parameters were studied in leaves of *Vigna radiata* at the interval of 15 days after each spray.

Determination of Chlorophyll and Carotenoid content: Fresh leaf samples were extracted with 80% acetone. For spectrophotometric determination of chlorophyll, a, chlorophyll b and carotenoid contents, the absorbance of the extracts were measured at 645 and 663 nm and 470 nm following the method by Lichtenthaler and Wellburn (1983) and calculated using the following formula:

Chlorophyll (a) in (mg/g) = 12.7(A663) – 2.69(A645) × V/1000 × W

Chlorophyll (b) in (mg/g) = 22.9(A645) – 4.68(A663) × V/1000 × W

Carotenoid (mg/g) = [1000(A470)-3.27 (Chl a)-104 (Chl b)]/229

Determination of Protein Content: The estimation

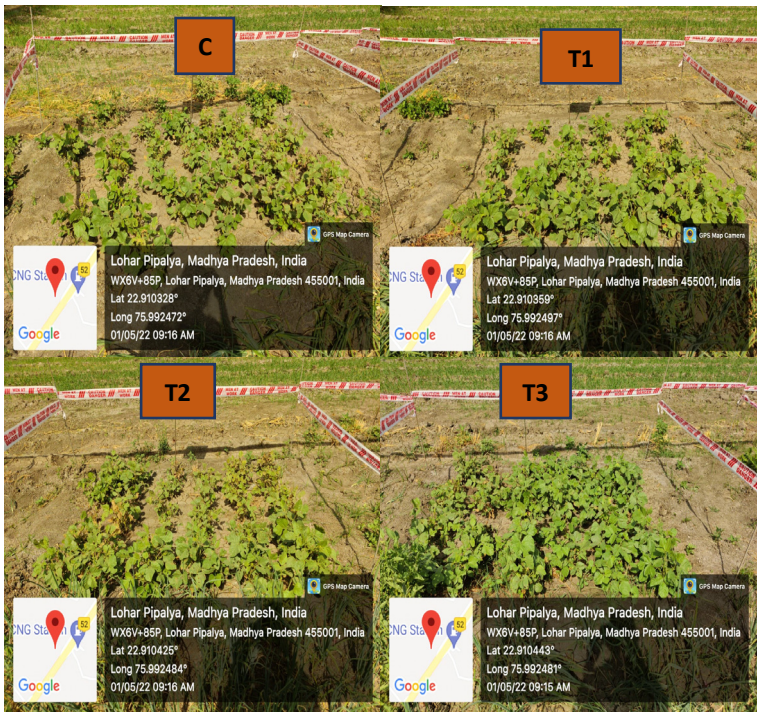

Fig. 1. Stimulatory effect of Bioenzyme on growth of mung bean

of protein was determined as per standard procedure given by Lowry *et al.* (1951). Protein content was estimated using a standard curve prepared with 40-200 µg of BSA at 660nm.

Determination of Carbohydrate Content: It was determined by the method as given Hedge *et al.* (1962). Carbohydrates are dehydrated by conc. H_2SO_4 to form furfural which condenses with anthrone to form a blue-green coloredcomplex which is measured colorimetrically at 630 nm.

Determination of Malondialdehyde Content: The level of lipid peroxidation as an indicator of oxidative stress was measured by using malondialdehyde (MDA), a decomposition product of the polyunsaturated fatty acid present in membrane lipids. It was estimated using thiobarbituric acid (TBA) as the reactive material and measuring absorbance spectrophotometrically at 532 nm.

The extinction coefficient of 155 mM^{-1} $cm^{-1}$was used following the method of Heath and Packer, (1968).

Determination of Proline: Proline was extracted using sulphosalysilic acid and measured using acid ninhydrin. Leaf tissues were homogenized in 3% sulfosalicylic acid and the homogenate was centrifuged at 3,000×g for 20min. The supernatant was treated with acid ninhydrin, boiled for 1 h, and then the absorbance was determined at 520 nm by Bates *et al.* (1973).

Results and Discussion

Photosynthetic pigments: Nitrogen deficiency adversely affects the chlorophyll concentration and photosynthetic activity which automatically results in the reduced growth of the plant. In the present study significant increase in chlorophyll a and b content were reported with supplementation of all the dilutions with 2 ml/L water bioenzyme giving best results (Table 1). Though no effect has been reported on carotenoid content in leaves after spraying bioenzyme at any of the concentration used in the study. Similar findings showed positive effect of bioenzyme spraying on photosynthetic pigments (Wahyudi *et al.,* 2009, Wulandavi and Susanti *et al.* 2012). Application of bioenzyme as biofertilizer also showed faster growth and better content of photosynthetic pigments in chick pea seedlings (Singh *et al.,* 2020). Positive effect of garbage enzyme was reported on chlorophyll a, b and carotene content of cowpea seedlings by Christopher and Ehijivwo

Table 1. Effect of foliar spray with bioenzyme on photosynthetic pigments

Treatments	Chlorophyll a (mg/g)			Chlorophyll b (mg/g)			Carotenoid (mg/g)		
	After 15 days of first spray	After 15 days of second spray	After 15 days of third spray	After 15 days of first spray	After 15 days of second spray	After 15 days of third spray	After 15 days of first spray	After 15 days of second spray	After 15 days of third spray
C(Without spray)	0.01 ± .001	0.025±.001	0.045±.004	0.018 ± .01	0.047±.001	0.046±.001	0.032 ± .002	0.017±.005	0.018±.001 ^{ns}
T1 (1 ml/L water)	0.023 ±.001** (130%)	0.028±.001** (12%)	0.057±.004** (27%)	0.022 ±.005*** (22%)	0.064±.002*** (36%)	0.082±.001*** (78%)	0.034 ±.001^{ns} (6%)	0.018±.005^{ns} (5%)	0.018±.005^{ns} (0%)
T2 (2 ml/L water)	0.027± .001*** (170%)	0.032±.002*** (28%)	0.070±.001** (55%)	0.029± .002*** (61%)	0.068±.002*** (45%)	0.087±.002*** (89%)	0.040± .002 ^{ns} (25%)	0.017±.005^{ns} (0%)	0.021±.001 ^{ns} (16%)
T3 (3 ml/L water)	0.018 ± .001** (80%)	0.030±.001** (20%)	0.054±.002** (20%)	0.025 ± .003*** (39%)	0.055±.003*** (17%)	0.071±.002*** (54%)	0.033 ± .002 ^{ns} (31%)	0.017±.001 ^{ns} (0%)	0.019±.005 ^{ns} (5%)

* Indicates p value < 0.05 and is significant, ** indicates p value < 0.01 and is highly significant, *** indicates p value< 0.001 and is extremely significant.

2021. The treatment of rice with ecoenzyme resulted in enhanced absorption of nitrogen from soil leading to increased chlorophyll content, better growth and improved yield (Barman *et al.*, 2022)

Biochemical parameters: Carbohydrate content determined after each spray of bioenzyme showed highly significant increase in all the treatment as compared to control (Table 2). Increase in photosynthetic pigments may have resulted in increased photosynthesis leading to enhanced carbohydrate content. Tazuke and Sakiyama (1991) in study on pepper fruit reported an increase in carbohydrate content after spraying of bioenzyme. The possible reason for increase in carbohydrate content may be enhancement in respiration capacity, subsequently there would be increase in metabolic activity and growth. Sethi *et al.* (2021) reported in a case study that use of ecoenzyme resulted in improved photosynthesis, nutrient and water uptake leading to improved quality and quantity of fruits and vegetables.

Significant increase was reported in protein content in leaves of mung bean after each spray as mentioned in Table 2. White *et al.* (2005) highlighted, that bioenzyme contains essential elements like zinc, iron, manganese, magnesium, sulfur and boron. Probably these elements involved in the activity of enzymes plays role in the protein biosynthesis. Higher amount of nitrogenous compound in the bioenzyme, accelerate the rate of biosynthesis of amino acids (Leegod *et al.*, 2000). Increase in protein content was reported in wheat with the application of garbage enzyme (Sethi *et al.*, 2021).

Oxidative stress parameters: In the open field plants are exposed to adverse environmental conditions produce free radicals in an excessive amount, can lead to increased production of ROS which can cause cell death by enhancing peroxidation of lipid, oxidation of protein, damage to nucleic acid, inhibition of important enzymes and initiation of pro-

Table 2. Effect of foliar spray with bioenzyme on biochemical parameters

Treatments	Carbohydrate (%mg)			Protein (mg/g)		
	After 15 days of first spray	After 15 days of first spray	After 15 days of first spray	After 15 days of first spray	After 15 days of second spray	After 15 days of third spray
C(Without spray)	2.0 ±0.4	2.0 ±0.4	2.0 ±0.4	2.59 ±0.05	3.22± 0.19	3.99± 0.12
T1 (1 ml/L water)	5.9 ±0.8** (195%)	5.9 ±0.8** (195%)	5.9 ±0.8** (195%)	2.93 ±0.1 (13.12%)	3.95± 0.26 (22.67%)	4.49± 0.12** (12.53%)
T2 (2 ml/L water)	7.46±0.7*** (273%)	7.46±0.7*** (273%)	7.46±0.7*** (273%)	3.95±0.2** (52.50%)	4.08± 0.28** (26%)	4.98± 0.1** (25%)
T3 (3 ml/L water)	4.73 ±1.1*** (136%)	4.73 ± 1.1*** (136%)	4.73 ±1.1*** (136%)	2.79 ± 0.06** (8%)	3.41± 0.43** (5.90%)	4.51± 0.12** (13.03%)

* Indicates p value < 0.05 and is significant, ** indicates p value < 0.01 and is highly significant, *** indicates p value< 0.001 and is extremely significant.

Table 3. Effect of foliar spray with bioenzyme on oxidative stress parameters

Treatments	MDA (nmoles/g)			Proline (µmoles/g)		
	After 15 days of first spray	After 15 days of first spray	After 15 days of first spray	After 15 days of first spray	After 15 days of second spray	After 15 days of third spray
C	0.063±.005	0.020±.003	0.024±.01	0.027 ± .001	0.021 ± .002	0.027±.001
T1 (1 ml/L water)	0.066 ±.005^{ns} (5%)	0.024±.005^{ns} (20%)	0.024±.009^{ns} (0%)	0.011 ± .0005*** (-59.25%)	0.013 ± .003* (-38.09%)	0.023 ± .003^{ns} (-14.81%)
T2 (2 ml/L water)	0.066± .005^{ns} (5%)	0.006±.002*** (-.028%)	0.026±.006* (8.33%)	0.011 ±.0005*** (-59.25%)	0.009 ± .001*** (-57.14%)	0.052 ± .004*** (92.59%)
T3 (3 ml/L water)	0.073 ± .005^{ns} (16%)	0.018±.001^{ns} (-10%)	0.021±.003^{ns} (-12.5%)	0.016 ± .001*** (-40.74%)	0.024 ± .001^{ns} (14.28%)	0.063 ± .011*** (133.33%)

* Indicates p value < 0.05 and is significant, ** indicates p value < 0.01 and is highly significant, *** indicates p value< 0.001 and is extremely significant.

Table 4. ANOVA between three different dilutions of bioenzyme

Parameters	F value between T1, T2, T3 After 15 days of first spray	F value between T1, T2, T3 After 15 days second spray	F value between T1,T2,T3 After 15 days third spray
Chlorophyll a	32.17***	3.79	27.87***
Chlorophyll b	7.4*	14**	67***
Carotenoid	9.6*	0.8	4.72
Carbohydrate	8.35*	15.8*	175.21***
Protein	42***	3.33	16.74***
MDA	1.33	94.04***	0.39
Proline	40***	36***	23**

Tabulated F value for 2,6 degree of freedom is 5.14. * Indicates p value < 0.05 and is significant, ** indicates p value < 0.01 and is highly significant, *** indicates p value< 0.001 and is extremely significant.

grammed cell death (Hasanuzzaman *et al.*, 2014). In the control may be the limiting amount of the nutrients in the soil create nutrient deficiency resulting in increased production of ROS. As mentioned in Table 3, MDA content was insignificantly affected in all the treatments after first foliar spray. Though significant decrease was observed after second spray only with 2 ml/L (T2) as compared to control. Decrease in lipid peroxidation measured by MDA content indicates increased tolerance of plant to changing environmental condition. Supplementation of bioenzyme fulfil the requirement of the limiting nutrients overcoming the nutrients deficiency stress hence reducing the ROS production and MDA content.

As shown in Table 3, highly significant decrease in proline content were observed after first spray of all the three dilutions of bioenzyme, however after third spray proline content was increased significantly corresponding to increased concentration of bioenzyme. Consistence with the present findings, in a review application of many biostimulants resulted in the improved antioxidative system as compared to control (Calvo *et al.*, 2014). Furthermore, Aydin *et al.*, (2012) observed that humic substance application under saline conditions increased proline content, and reduced membrane leakage and reactive oxygen species (ROS) generation in the common bean (*Phaseolus vulgaris* L.), reflecting better adaptability to saline conditions. Probable reasons for that may be humic acid content present in bioenzyme has increased leaf hydration under dry soil conditions as well as root growth, shoot growth, and antioxidant capacity (Zhang *et al.*, 2002 and 2008). Bioenzyme through its positive effect on proline content, reduces ROS level and maintains cellular homeostasis (Garcia *et al.*, 2012).

Conclusion

The study highlights the positive effect of bioenzyme application on photosynthetic pigments, biochemical and oxidative stress parameters. This research concludes that the ecofriendly product bioenzyme can be a good alternative for chemical fertilizers to protect soil and our ecosystem. Bio-enzymes helps to reduce some waste & turn into a useful substance to the society which is economical and cheaply available and the end product can be completely useful.

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Conflict of Interest

All the authors hereby declare that there is no conflict of interest regarding the publication of this article.

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