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Biochemical response of mungbean (*Vignaradiata* L.) under the influence of reduced dose of chemical fertilizer and different time and method of application of biofertilizer

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Fundamental differences between organic and conventional production systems, particularly in soil fertility management, may affect the nutritive composition of plants, including secondary plant metabolites. The availability of inorganic nitrogen in particular has the potential to influence the synthesis of secondary plant metabolites, proteins, and soluble solids. This study was conducted to evaluate the on-farm assessment of biochemical changes in leaves of mungbean by employing different varieties and different treatments such as different reduced doses of N:P:K chemical fertilizer, different method and time of application of biofertilizer. Untreated seeds were used as control. PDM-11 variety showed higher accumulation of chlorophyll, sugar, protein in leaves and PUSHA-9531 reported higher accumulation of amino acid content in leaves. Significant level of variation was observed in the level of plant metabolites and macromolecules in seeds among the six studied mungbean varieties.

Key words: Biochemistry, Mungbean, Phenol, Protein

Introduction

Mung bean (*Vignardiata* L.) is a leguminous pulse crop which used is as a vegetable protein source, animal fodder and green manure, it contains isoflavonoids having estrogen and antioxidant activities that used in prevention of many diseases such as cancer, and it also exhibits antimicrobial and insecticidal activities (Aggrawal*et al*., 1997). Imbalanced and continuous use of chemical fertilizers in cropping systems is leading to imbalance of nutrients in soil which have an adverse effect on soil health and also biochemical constituents of plant. The productivity of any crops depends on the process of photosynthesis, which in turn depends on the chlorophyll content of leaves in plants (Shah, 1959).

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Fundamental differences between organic and conventional production systems, particularly in soil fertility management, may affect the nutritive composition of plants, including secondary plant metabolites. The availability of inorganic nitrogen in particular has the potential to influence the synthesis of secondary plant metabolites, proteins, and soluble solids. Stamp (2003) reported that increased crop growth and development rates and greater biomass accumulation in well-fertilized crops would also correlate with decreased allocation of resources toward the production of starch, cellulose, and non-nitrogen-containing secondary metabolites. Phenolic compounds are important group of bioactive compounds is that of. These are widely distributed in fruits, herbs and vegetables and their antioxidant activity has been extensively reported. Nitrogen is the nutrient that has the largest effect on the physiology and yield of cotton plants. It is an essential nutrient for production of plant dry matter, as well as many energy-rich compounds that regulate photosynthesis and plant production (Wu *et al.*,1998) Nitrogen is also vital in protein structure and in basic N compounds such as purines, pyrimidines, porphyrins and coenzymes. Purines and pyrimidines are constituents of the nucleic acids RNA and DNA, which are essential for protein synthesis. Porphyrin is found in metabolically important compounds such as chlorophylls and the cytochrome enzymes, which are necessary for photosynthesis and respiration (Devlin, 1972). Form of nitrogen fertilizer could have on important influence on the accumulation of chlorophyll in plants. Lettuce plants fertilized with two form of nitrogen (NO_3 + NH_4) had significantly more chlorophylls *a* and *b* than that fertilized with nitrate nitrogen; and plants fertilized with ammonium nitrogen had the highest concentration of chlorophylls (Michałek and Rukasz, 1998).

Nitrogen fertilization stimulates accumulation of chlorophyll. Mihailovic*et et al*., (1997) reported that ammonium ions in nutrient solution increased concentration of chlorophylls *a* and *b* in wheat leaves in comparison with nitrate ions. There is an optimal relationship between plant N content and CO_2 assimilation (Sinclair and Horie, 1989).

Interest in other plant secondary compounds has increased because of their potential effects on improving human health (Dumus *et al*., 2003) Phenolic compounds play a role in plant defence mechanism to resist diseases and insects, also acts as antioxidants if consumed in food. Higher levels of phenolic compounds frequently have been reported in organically grown crops than in conventionally grown crops (Carbonaro*et al*., 2002). Some earlier workers (Michałek and Rukosz, 1998) found that ammonium nitrogen increased accumulation of phenols. Influence of ammonium and nitrate nitrogen applied together on phenol concentration depended on cultivar. Some earlier workers (Rozek*et al*., 1994) reported that fertilization with reduced form of nitrogen increased content of sugars in leaves of lettuce in comparison to plants fertilized only with NO_3. The same effect was observed in corn salad leaves in the case of second growing period. The aim of the study was to investigate the biochemical changes in leaves and seeds of mungbean plants associated with different reduced dose of chemical fertilizer and supplement by the biofertilizer and different methods of biofertilizer application and subsequent grain yield.

Materials and methods

Field experiments were conducted during summer season of 2003-04, 2004-05, and 2005-06 at Crop Research and Seed Multiplication Farm of Burdwan University, India (Latitude 67^0 50’ 51’’ N and longitude 23^0 15’ 12’’ E). The source of chemical fertilizer includes urea as nitrogen fertilizer, single super phosphate as phosphate and muriate of potash as potassium. Pure culture of *Rhizobium* was used as bacterial biofertilizer and was procured from Exploration India, Research and Development Wing, Burdwan Insitute of Management and Computer sciences, Burdwan, West Bengal, India. In 2003-04, five varieties of mungbeen [V_1-NORMAL, V_2-PDM-11, V_3-PDM-54, V_4-PDM-139, V_5-PUSHA-9531 and V_6-PANT MUNG] were cultivated under recommended dose of chemical fertilizer (Directorate of Agriculture, Government of India). In 2004-05 the treatment combination includes.

During the summer season (2004) we conducted one field experiment with different reduced dose of nitrogen(N) and phosphate(P) fertilizer along with recommended dose of potassium(K) which includes T_1-20% less nitrogen + 25% less phosphate + recommended dose of potassium; T_2-30% less nitrogen + 25% less phosphate + recommended dose of potassium; T_3-40% less nitrogen + 25% less phosphate + recommended dose of potassium; T_4-50% less nitrogen + 25% less phosphate + recommended dose of potassium; T_5- 60% less nitrogen + 25 % less phosphate + recommended dose of potassium and T_6-recommended dose i.e. 20:40:20.

In 2005-06 the treatment combination was T_1-as basal application 1.5 kg ha^{-1} + on 21 DAS at 0.75 kg ha^{-1} as spray + on 42 DAS at 0.75 kg ha^{-1} as spray; T_2- basal application 3 kg ha^{-1}; T_3-as basal application at 1.5 kg ha^{-1} + on 21 DAS at 1.5 kg ha^{-1} as soil application; T_4-as basal application at 1.5 kg ha^{-1} + on 21 DAS at 1.5 kg ha^{-1} as spray; T_5-as basal at 0.75 kg ha^{-1} + on 21 DAS at 1.5 kg ha^{-1} as soil application + on 42 DAS at 0.75 kg ha^{-1} as soil application. The experiment was curried out in a randomized block design with different treatment in different year with three replications. The experiments were conducted in randomized block design with three replications. Individual plot size (5mx5m); plant row to row spacing 30 cm and plant to plant 15 cm.

For biochemical estimation in leaves and seeds constituents such as total chlorophyll (Arnon, 1949), total soluble sugar (McCready*et al.*, 1952), total amino acids(Moore and Stein, 1948) , total protein (Lowry *et al*., 1950) was estimated from physiologically active leaves and other parameters like RNA, DNA (Choudhuri and Chatterjee, 1970). Ascorbic acid (along with total soluble sugar (McCready*et al*., 1950), total amino acid(Moore and Stein, 1948), and total protein (Lowry *et al*., 1950) was estimated from seeds.

After harvesting the biomass (as dried) of the whole plant were estimated. Then the grain yields were estimated in square meter and again it is calculated in hector.

Physiologically active leaf (3^{rd} and 4^{th} from the top) samples from ten different plants of each plots were plucked randomly for determination of different biochemical parameters. Composite samples of leaf were crushed separately in a mortar with 10 ml of distilled water, poured in centrifuge tube and centrifuged at 10000 rpm for ten minutes. The supernatant was used for soluble sugar determination following the method of Mc.cready*et al.*, (1950); for estimation of free amino acid in leaf samples Moore and Stain (1948); for chlorophyll content ($mg.g^{-1}.f.w$) method of Arnon (1949); for protein content ($mg.100g^{-1}$) method of Lowry *et al*., (1951).

Seeds of were imbibed in distilled water under room temperature for overnight. After that seed coat was removed and the seeds were crashed in a mortar and pestle along with distilled water and centrifuged for 10000 rpm for 10 minutes. The supernatant was used for soluble sugar determination following the method of Mc.cready*et al*., (1950). For determination of free amino acid in seeds Moore and stain (1948); for protein (Lowry *et al*., 1951); for Ascorbic acid (Mukherjee and Choudhury, 1984); for nucleic acid (Choudhuri and Chatterjee, 1970) standard methods were followed.

All the experimental results of the subsequent three years were analysed by one way analysis of variance (ANOVA). All the statistical analysis were carried out with the programme SPSS 11.0 for windows. All values were expressed as mean values. The statistical significance of difference between the different treatments were established using Duncan's Multiple Range Test (DMRT).

Results

This study pertained to assess the biochemical changes in leaves and seed of mungbean under different treatments of reduced dose of chemical fertilizer and different methods and time of application of biofertilizer and was evaluated in terms of chlorophyll, protein, amino acid, sugar content in leaves and accumulation of chlorophyll, protein, amino acid, sugar, ascorbic acid, phenol, DNA and RNA for enhanced crop growth , metabolism and optimum protection against pathogens and pest.

In 2003, there was significant level of variation in the level of total chlorophyll in the leaves of six mungbean varieties. Minimum and maximum total chlorophyll levels were observed in V_5 and V_3 respectively (Table-1). In 2004, 30% reduction of the recommended dose of nitrogen fertilizer (T_2) was stimulatory for chlorophyll content in leaves when compared to application of recommended dose of chemical fertilizer (T_6). During 2005, soil application of biofertilizer was found to promote the total chlorophyll content in leaves of mungbean plants (T_3 and T_6) over spray application (Table-2).

In 2003, there was significant level of variation in the level of total chlorophyll in the leaves of six mungbean varieties. Minimum and maximum total chlorophyll levels were observed in V_6 and V_3 respectively (Table-1). In 2004, 30% reduction of the recommended dose of nitrogen fertilizer (T_2) significantly promoted the accumulation of sugar content in leaves when compared to application of recommended dose of chemical fertilizer (T_6). During 2005, soil application of biofertilizer significantly promoted the accumulation of soluble sugar content in leaves of mungbean plants (T_3 and T_6) over spray application (Table-2).

Significant levels of variation in the level of amino acid in the leaves of six mungbean varieties were observed during the present investigation of 2003. Highest and lowest accumulation of amino acid were found to be in V_3 and V_6 respectively (Table-1). In 2004, 30% reduction of the recommended dose of nitrogen fertilizer (T_2) was found to be stimulatory towards biosynthesis of amino acid in leaves when compared to application of recommended dose of chemical fertilizer (T_6). Soil application of biofertilizer at different phases of crop growth were found to be stimulatory towards biosynthesis of amino acid and its accumulation in leaves of mungbean plants when compared to spray application (Table-2).

Protein content in leaves varied significantly among the seven varieties of mungbean. Maximum and minimum accumulations were recorded by varieties V_6 and V_3 respectively (Table-1). In 2004, 30% reduction of the recommended dose of nitrogen fertilizer (T_2) was found to be stimulatory towards biosynthesis of protein in leaves when compared to application of recommended dose of chemical fertilizer (T_6). Soil application of biofertilizer at different phases of crop growth were found to be stimulatory towards biosynthesis of protein and its accumulation in leaves of mungbean plants when compared to spray application (Table-2).

The sugar content in seeds of six studied mungbean varieties showed significant variation among them. Maximum and minimum accumulation of sugar was found to be in V_3 and V_6 variety respectively (Table-3). In 2004, 30% reduction of the nitrogen fertilizer dose was found to be stimulatory towards accumulation of sugar in freshly harvested seeds when compared to application of recommended dose of chemical fertilizer. Soil application of biofertilizer significantly promoted accumulation of sugar in seeds of freshly harvested mungbean (Table-4).

The amino acid content in freshly harvested seed varied between 15.303 g $100g^{-1}$ in V_3(PDM-54) and 31.80 g $100g^{-1}$ in V_6 (PANT MUNG) during 2003-04.(Table-3). The amino acid content in seeds decreased proportionately as the rate of nitrogen fertilizer reduction increased gradually. Soil application of biofertilizer at different rate and time was found to be stimulatory towards amino acid biosynthesis leading to higher accumulation of amino acid in seeds over spray application (Table-4).

Significant levels of variation in the level of protein in the seeds of six mungbean varieties were observed during the present investigation of 2003. Highest and lowest accumulation of amino acid were found to be in V_3 and V_6 respectively (Table-3). 30% reduction of nitrogen fertilizer dose was found to be stimulatory over application of recommended dose of nitrogen fertilizer towards protein synthesis in mungbean seeds. Soil application of biofertilizer significantly promoted better protein synthesis in mungbean seeds leading to higher protein content in seeds (Table- 4).

\Ascobic acid content in seeds showed significant level of variation among the six studied mungbean varieties in 2003. PDM-54 seeds recorded the highest accumulation of ascorbic acid.(Table-3). Treatment T_2 recorded highest accumulation of ascorbic acid in mungbean seeds during 2004.Spray application of biofertilizer at different rate and time of crop growth was found to be inhibitive towards accumulation of ascorbic acid in mungbean seeds (Table-4).

Phenol content in seeds showed significant level of variation among the six studied mungbean varieties in 2003. PDM-54 seeds recorded the highest accumulation of ascorbic acid (Table-3). Treatment T_2 recorded highest accumulation of phenolics in mungbean seeds during 2004.Spray application of biofertilizer at different rate and time of crop growth was found to be inhibitive towards accumulation of phenolics in mungbean seeds (Table-4).

Nucleic acid (DNA and RNA) content in mungbean seeds varied significantly among the six varieties. The level of ribonucleic acid (RNA) content in seeds was higher than deoxy-ribonucleic acid(DNA) among the six studied varieties (Table-3). Nucleic acid content in seeds varied significantly along with different nitrogen fertilizer reduction dose in 2004 as well as with the method, rate and time of application of biofertilizer. The RNA content in seeds was higher than DNA for both the years of 2004 and 2005 (Table-4).

Discussion

The significant variation in the level of total chlorophyll content in physiologically active leaves of six mungbean varieties may be due to variable rate of biosynthesis of chlorophyll and photosynthesis depending upon the genetic potential of the mungbean varieties.Total chlorophyll increased in T_2 treatment than the recommended dose which might be due to significant influence of microorganisms as biofertilizer of producing growth promoting substances resulting in more efficient absorption of nutrients, which main components of photosynthetic pigments and consequently the chlorophyll content was increased (Gomaa and Abou-Aly, 2001). Similar results were obtained by Hassan (2009).

The soil application of bacterial biofertilizer at different phases of crop growth may have promoted increased chlorophyll content in leaves due to the supply of higher amount nitrogen to the growing tissue and organs supplied by N_2 fixing *Rhizobium.* (Chandrasekhar *et al.,* 2005) over spray application.

Total soluble sugar content in leaves showed considerable variation among the different mungbean varieties which may be attributed towards the variable rate of photosynthesis, leading to production of variable amount of photosynthate among the different varieties. The sugar content in leaves increased upto 30% of reduced dose of nitrogenous chemical fertilizer and then decreased further with gradual increment of reduction of nitrogen fertilizer dose which may be attributed towards higher rate of translocation of sugar transported from leaves to flowering parts and its subsequent utilization for the development of seeds (Chandrasekar BR, 2005) under reduced supply of nitrogen to crop plants. This therefore indicates the role of nitrogen towards biosynthesis and accumulation of sugar in crop plants. Similar findings were reported by Setua *et al*. (2005) in mulberry leaves. The results also reveals that dual application of biofertilizers and chemical fertilizer has pronounced influence on biosynthesis of carbohydrates in the leaves in terms of soil application over spray at different phases of crop growth (Rao*et al*., 2007).

Significant variation in the level of amino acid content in leaves among the different varieties may be attributed towards the differential rate of inorganic nitrogen assimilation towards nitrogen transporting amino acid which are used to transfer nitrogen from source organs to sink tissues and to build up reserves during periods of nitrogen availability for subsequent use in growth, defence and reproductive processes. The results suggest that higher reduction of recommended dose of nitrogen fertilizer may be inhibitive towards biosynthesis of amino acid in leaves due to lesser assimilation of inorganic nitrogen transporting amino acid which are used to transfer nitrogen from source organs to sink tissues and to build up reserves during periods of nitrogen availability for subsequent use in growth, defence and reproductive processes. Soil application of biofertilizer was found to be stimulatory over spray application towards biosynthesis of amino acid which indicates lesser availability of nitrogen under non-rhizospheric (spray) application.

Significant level of variation in the level of total soluble protein content in leaves of different varieties of mustard under the field trial of 2003 reveals differential rate of nitrogen assimilation by crop plants. The protein content in leaves significantly reduced from 40% reduction of recommended dose of nitrogen fertilizer (T_3) onwards due to lesser supply of nitrogen towards protein synthesis in leaves. Soil application of biofertilizer was found to be superior over spray application in terms of protein content in leaves at different stages of crop growth which might be due to optimum supply of precursor molecule for protein synthesis in crop plants under rhizospheric application (soil application) of biofertilizer.

Significant level of variation in total soluble sugar content in seeds of mungbean varieties may be attributed towards the variable rate of photosynthesis leading to variable rate of accumulation of sugar amount in seeds of six munbean varieties. During 2004,soluble sugar in seeds increased up to T_2 treatment and then decreased gradually which might be attributed towards utilization of sugar molecules in TCA cycle for amino acid protein synthesis in plant cell. Our findings were similar with earlier findings of Almodares et al., (2009). During 2005, the soil application of biofertilizer at different stages of crop growth was found to be stimulatory over spray application which might be due to higher availability of nitrogen under soil application. This may have increased the vigour of mungbean plants and therefore promoted increased leaf area with higher synthesis of assimilates due to enhance rate of photosynthesis. Such effects have attributed to increased rate of translocation of photosynthetic products from leaves to developing fruits, there by increasing total sugar. Such enhancement of sugar was noted by Magge, (1963) in strawberry plants.

During 2003, the protein content in seeds showed significant variation among the six studied mungbean varieties which might be due to variable rate of protein synthesis in developing seeds under the influence of variable rate of nutrient uptake from the soil by the mungbean varieties. Protein content in seeds reduced significantly with gradual enhanced rate of reduced dose of nitrogen fertilizer as nitrogen is the precursor molecule for protein synthesis. Soil application of biofertilizer promoted greater availability of nitrogen to mungbean crop plants under the influence of symbiotic nitrogen fixation by the applied *Rhizobium* strain resulting into higher accumulation of protein in mungbean seeds.Our findings were similar with the earlier findings of Selvakumar*et al*., (2009). Higher availability of nitrogen under soil application of biofertilizer increases amino acid synthesis in the leaves and this stimulates the accumulation of protein in the seed (Patil*et al*., 1997).

Amino acid content in seeds showed significant variation among the six studied mungbean varieties indicating towards variable rate of nitrogen uptake by the mungbean varieties. The reduced accumulation of amino acid content in seeds under different reduced dose of nitrogen fertilizer may be due to lesser availability of nitrogen leading to reduced biosynthesis of amino acids in crop plants. Soil application of biofertilizer significantly improved nitrogen availability to crop plants and therefore promoting increased amino acid synthesis in leaves leading to higher accumulation of amino acid in seeds through translocation (Ram Rao*et al*, 2007).

Ascorbic acid content in seeds of six studied mungbean varieties varied significantly due to variability in the antioxidant property of different varieties under oxidative stress condition. Low dose of nitrogen means low nitrogen mineralization in soil and decrease vitamin-C content in seeds (Tuncay*et al.*, 2004). Elevated level of ascorbic acid content in seeds of mungbean under different rate and time of soil application of biofertilizer may be due to higher availability of nitrogen which promoted the ascorbic acid content in seeds. Our findings are with the line of earlier findings of Bhat, (1999) in strawberry.

The variation in the level of phenolics in seeds of six studied mungbean varieties may be due to growing environment, season and cultivar with cultivar differences being most significant (Zhao *et al.*,2007). With reduction dose of chemical fertilizer phenol content decreased significantly. The similar observation was noted by Alizadeh*et al*., (2010). They are found that fertilizer can effect on total phenolic compound in *Saturejahortensish*(Lamiaceae). Higher phenol content in mungbean seeds under the influence of soil application of biofertilizer over spray application might be attributed towards the use of accumulated nitrate in plant thus enabling it to use more carbohydrates for structural growth.(Hanafy Ahmed *et al.*, 2000).

The variation of DNA content in all the six varieties is due to genetic make up of the crop varieties. The nucleic acid content (both DNA and RNA) reduced significantly with higher rate of nitrogen fertilizer reduction dose as biosynthesis of basic nitrogenous compounds such as purines, pyrimidines were inhibited under reduced supply of nitrogen (Devlin, 1972).The soil application of biofertilizer were found to be stimulatory towards availability of nitrogen to crop plants and biosynthesis of purines and pyrimidines leadimg to higher nucleic acid (both DNA and RNA) content in mungbean seeds (Devlin, 1972). Nucleic acid (DNA and RNA) contents were much higher obtained from plants treated with biofertilizer. This may be due the active synthesis of nucleic acids concurrently with decreasing the hydrolytic and oxidative enzyme activities. Furthermore, it is known that auxins, gibberellins, and cytokinins stimulate the synthesis of nucleic acids and inhibit the activities of RNase, DNase and 3-nucleotidase (Steward, 1991). RNA content in seeds was found to be higher than DNA content.

Correlation study revealed that only protein and sugar in 2004-05 and amino acid and chlorophyll in 2005-06 in mungbean leaves showed a positive correlation at 5% level of significance. Moreover in 2004, mungbean seeds sugar shows positive relationship with phenol and DNA at 1% level of significance. Ascorbic acid showed a positive correlation with amino acid; protein at 0.05 level of significance and with RNA at 1% level of significance. Phenol level in seeds showed positive correlation with protein and DNA at 5% and 1% level of significance respectively. Ascorbic acid showed a positive relationship with protein and RNA at 5% level and 1% level of significance respectively. In 2004, under subsequent reduction of nitrogen fertilizer dose amino acid was found to be positively correlated with protein, ascorbic acid, DNA and RNA at 1% level of significance. Similarly ascorbic acid and protein individually showed positive correlation at 1% level of significance with RNA and DNA. During 2005, a positive correlation exists between sugar and DNA, RNA and amino acid with protein and RNA at 1% level of significance (Table-5).

Table 1. Biochemical contents of leaves of six mungbean varieties during experimental field trial of 2003

Variety	**Chlorophyll ($mg.g^{-1}f.w$)**	**Soluble sugar (g/100g)**	**Amino acid (g/100g)**	**Protein (g/100g)**
V_1	0.206^{bc}	22.91^{b}	35.49^{a}	36.52^{bc}
V_2	0.272^{ab}	23.71^{d}	35.49^{ab}	36.96^{b}
V_3	0.326^{a}	24.91^{bc}	40.19^{a}	41.68^{a}
V_4	0.130^{c}	22.42^{a}	32.66^{bc}	34.23^{d}
V_5	0.074^{de}	21.40^{e}	24.46^{d}	30.87^{e}
V_6	0.120^{de}	17.91^{f}	23.40^{e}	30.25^{e}

Note: Means followed by the same letter withina treatment were not significantly different at 5% level using Duncan's multiple range.test (DMRT). Means of three replicates were taken.

Table 2. Biochemical contents of leaves of mungbean (PDM-54) during experimental field trials of 2004 and 2005

Treatments	Chlorophyll ($mg.g^{-1}f.w$)		Soluble sugar (g/100g)		Amino acid (g/100g)		Protein (g/100g)	
	2004	2005	2004	2005	2004	2005	2004	2005
T_1	1.094^{e}	0.761^{ad}	18.96^{f}	30.46^{e}	20.85^{e}	33.62^{ce}	36.69^{de}	40.26^{e}
T_2	1.810^{a}	0.673^{ac}	44.00^{a}	39.36^{d}	25.78^{a}	35.63^{cd}	40.17^{a}	40.84^{bcd}
T_3	1.326^{cd}	0.916^{ab}	38.40^{bc}	42.12^{ab}	24.84^{bc}	40.65^{b}	38.12^{bc}	42.99^{ab}
T_4	1.254^{cde}	0.867^{ac}	23.56^{d}	40.23^{c}	24.18^{cd}	36.03^{c}	37.59^{cd}	42.64^{abc}
T_5	1.476^{bc}	1.062^{a}	17.02^{e}	42.82^{a}	19.38^{f}	43.38^{a}	33.22^{f}	43.68^{a}
T_6	1.583^{ab}		38.41^{bc}		25.64^{ab}		38.96^{b}	

Note: Means followed by the same letter within a treatment were not significantly different at 5% level using Duncan's multiple range. test (DMRT). Means of three replicates were taken.

Table 3. Biochemical contents in freshly harvested seeds of six mungbean varieties during experimental field trial of 2003

Varieties	Soluble sugar (g/100g)	Amino acid (g/100g)	Protein (g/100g)	Phenol (μg/g)	Ascorbic acid (mg/100g)	DNA (mg/100g)	RNA (mg/100g)
V_1	7.61^{bc}	24.60^{bc}	28.22^{bc}	751^{c}	8.61^{c}	482.35^{c}	766.92^{c}
V_2	8.22^{b}	25.30^{b}	29.54^{ab}	906^{b}	11.20^{b}	510.97^{b}	888.57^{ab}
V_3	9.30^{a}	31.80^{a}	29.66^{a}	947^{a}	13.13^{a}	547.47^{a}	895.01^{a}
V_4	6.60^{d}	22.90^{d}	28.09^{cd}	623^{d}	8.03^{cd}	397.15^{de}	744.97^{d}
V_5	6.33^{e}	18.50^{e}	26.51^{e}	620^{de}	7.65^{df}	394.66^{de}	724.74^{e}
V_6	5.01^{f}	15.30^{f}	17.54^{f}	605^{f}	7.51^{f}	243.32^{f}	984.73^{e}

Note: Means followed by the same letter within a treatment were not significantly different at 5% level using Duncan's multiple range.test (DMRT). Means of three replicates were taken.

Table 5. Correlation between different biochemical variables in leaves of mungbean plants during 2003,2004 and 2005

Year of Experiment	Variables	r-value	Levels of significance
2003(leaf)			No significance correlation was found
2004(leaf)	Protein and sugar	0.762	0.05
	Sugar and chlorophyll	-0.897	0.01
	Amino acid and chlorophyll	0.756	0.05
	Amino acid and sugar	-0.822	0.01
2003(seed)	Phenol and sugar	0.793	0.01
	Sugar and DNA	0.925	0.01
	Ascorbic acid and amino acid	0.476	0.05
	DNA and amino acid	-.565	0.05

	Phenol and protein	0.461	0.05
	Ascorbic acid and protein	0.572	0.05
	RNA and protein	0.549	0.05
	Phenol and DNA	0.668	0.01
	Ascorbic acid RNA	0.917	0.01
2004(seed)	Protein and sugar	-0.463	0.05
	Ascorbic acid and sugar	-.844	0.01
	Protein and amino acid	0.865	0.01
	Ascorbic acid and amino acid	0.655	0.01
	DNA and amino acid	0.955	0.01
	RNA and amino acid	0.865	0.01
	Ascorbic acid and protein	0.796	0.01
	DNA and protein	0.681	0.01
	RNA and protein	0.695	0.01
	DNA and ascorbic acid	0.527	0.05
	RNA and ascorbic	0.679	0.01
2005(seed)	DNA and Sugar	0.609	0.01
	RNA and sugar	0.638	0.01
	Protein and amino acid	0.909	0.01
	Ascorbic acid amino acid	-.887	0.01
	RNA and amino acid	0.556	0.01
	Ascorbic acid and protein	-0.946	0.01
	Ascorbic acid and phenol	-0.447	0.05
	DNA and phenol	-0.756	0.01
	RNA and phenol	0.675	0.01

Conclusion

This study concludes that reduced dose of chemical fertilizer and different method and rate of application of biofertilizer reflects the variable biochemical response of mungbean plants towards synthesis and accumulation of simple organic molecules such as sugar, free amino acid, chlorophyll and protein in leaves and sugar, free amino acid, phenol, ascorbic acid , nucleic acid in seeds which reflects crop plant metabolism leading to better crop growth and yield. Higher reduction in the recommended dose of nitrogen fertilizer lead to inhibition of biosynthesis and accumulation of chlorophyll, sugar , protein , ascorbic acid , phenol and nucleic acid (both DNA and RNA) in leaves and seeds respectively. Although 30% reduction of nitrogen fertilizer dose and soil application of biofertilizer (T_3 and T_5) were found to be stimulatory towards accumulation of biological macromolecules in leaves and seeds as well as accumulation of secondary plant metabolites such as phenolics. From this it can be interpreted that chemical fertilizer reduction upto a certain level and soil application of biofertilizer is a suitable strategy in regulating plant metabolism leading to accumulation of certain metabolites for optimum plant growth along with enhanced plant defence system against insect and disease attack as well as through antioxidant defence mechanism which may adversely effect the growth and yield attributes of crop plants under agroclimatic condition of old alluvial soil, Burdwan, India. Nonetheless, more research efforts and participatory evaluation of the effects of reduction of chemical fertilizer dose and soil application of biofertilizer on biochemical response of crop plants are needed in this area.

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References

Aggrawal, R.K.; Kumar, P. and Power, J.F. (1997). Use of crop residue and manure to conserve water and enhance nutrient availability and pearl millet yields in an arid tropical region. Soil and Tillage Research 41:43-51.

Alizadeh, A., Khoshkhui, M., Javidnia, K, Firuzi, O., Tafazoli, E. and Khalighi, A. (2010). Effects of fertilizer on yield, essential oil composition, total phenolic content and antioxidant activity in *Satureja hortensis* L. (Lamiaceae) cultivated in Iran. Journal of Medicinal plants Research 4(1):33-40.

Almodares, A., Jafarinia, M. and Hadi, M.R. (2009). The effects of nitrogen fertilizer on chemical compositions in corn and sweet sorghum, American-Eurasian J. Agric. And Env.Sc 6(4):441-446.

Antipchuk, A.F., Tantsyurenko, E.V., Mantselyaruk, R.M. (1982). Effect of bacteria on tomato yield and quality. Teknologiya Pr-va-i-Effectivnost Primeneniya-Bakterialynkh-Udobreni 98-103. Fide Horticultural Abstracts 54:1768.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplast. Polyphenol oxidase in *Beta vulgaris*. Plant Physiology 24:1-15.

Bhat, N.H. (1999). Response of strawberry cultivars to varied levels of organic manure. M.Sc. thesis submitted to Sher-eKashmir university of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar.

Carbonaro, M., Mattera, M., Nicoli, S.,Bergamo, P.,Capelloni, P. (2002). Modulation of antioxidant compounds in organic vs conventional fruit (peach, *Prunus persica* l and pear *Pyrus communis* , L). J.Agr.Food.Chem. 50:5458-5462

Chandrasekhar, B.R., Ambrose, G., Jayabalan, N. (2005). Influence of biofertilizers and nitrogen source level on the growth and yield of *Echinochloa frimentacea* (Roxb.) Link., Journal of Agricultural Technology.

Choudhuri, M.A., Chatterjee, S.K. (1970). Seasonal changes in the level of some cellular components in the abscission zone of coleus leaves of different ages. Ann. Bot. 34:275-287.

Devlin, RM. (1972). Plant Physiology, 2nd edn. Padmanabhan for Affiliated East–West Press Pvt. Ltd, New Delhi, India.

Dumus, Y., Dadomo, M., Lucca, G. Di. And Groiler, P. (2003). Review: Effects of environmental factors and agriculture techniques on antioxidant content of tomatoes . Journal of Science Food and Agriculture 83:369-382.

Gomaa, A.O. and Abou-Aly, H.E. (2001). Efficiency of biofertilizer in the presence of both inorganic and organic fertilizers on growth, yield and chemical constitutents of anise plant (*Pimpinella anisum* L.). Proc. Of the fifth Arabian Hort. Cong. Ismailia, Egypt, pp. 73-80.

Hanafy ahmed, A.H., Mishriky, J.F. and Khalil, M.K. (2000). Reducing nitrate accumulation in lettuce (*Lactica saliva* L) plants by using different biofertilizers .ICEHM2000,Cairo University, Egypt, pp. 509-517.

Hassan, F. (2009). Response of Hibiscus sabdariffa L. Plant to some biofertilizer treatments. Annals Agric. Sci. Ain Shans Univ. Cairo 54:437-446.

Lowry, O.H., Rose Brough, N.J., Fan, A.L. and Randal, R.J. (1951) Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193:265-275

Magge, D.H. (1963). The reduction in growth in apple trees brought about by fruiting. J. Hort. Sci 338:119-125.

Mc Cready, R.M., Guggolz, J., Silviera, V. and Owens, H.S. (1950) Determination of starch and amylase in vegetables. Analytical Chemistry 22: pp. 1156.

Michalek, W. and Rukasz, I. (1998.) Wpływ zywienia azotowego i wybranych regulatorów wzrostu na zawartosc chlorofilu i fenoli w lisciach sałaty. Zeszyty Naukowe Akademii Zeszyty Naukowe Akademii Rolniczej Zeszyty Naukowe Akademii Rolniczej.

Mihailovic, N., Lazarevic, M., Dzeletovic, Ž., Vuckovic, M. and Durdevic, M. (1997). Chlorophyllase activity in wheat, *Triticum aestivum* L. leaves during drought and its dependence on the nitrogen ion form applied. Plant Science 129:141-146

Moore, S. and Stein, W.H. (1948). In Methods in Enzymol (Eds. Colwick S. P. and Kaplan N. D).Academic Press, New York 3: pp.468.

Mukherjee, S.P. and Choudhury, M.A. (1983) Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings Plant Physiology 58:166-170.

Patil, D.B., Naphade, K.T., Wankhade, S.G., Wanjari, S.S. and Potdukhe, N.R. (1997) Effect of nitrogen and phosphate levels on seed protein and carbohydrate content of cotton cultivars . Ind. J. Agric. Res. 31:133-135.

Prasad, F.M., Lal, A.M., Jilani, A.A., Sing, J.P., Arnold, R., Prasad, V.M. and Sing., R.R. (2006) Effect of different levels of Bioneema and Nitrogenous Fertilizer on Bio Chemical constituents in Tomato (*Lycopersicon esculentum* Mill.) c.v. Money maker, World Journal of Chemistry 1(1):28-29.

Rao, R.D.M., Kodandaremaiah, J., Reddy, M.P., Katiyar, R.S. and Rahmathulla, V.K. (2007). Effect of VAM fungi and bacterial biofertilizers on mulberry leaf quality and silkworm cocoon characters under semiarid conditions. Caspian Journal of Environmental Science. 5(2):111-117.

Rozek, S., Leja, M., Myeyezkowski, J. and Mareczek, A. (1994). The effect of fertilization with different forms of nitrogen on greenhouse lettuce quality and its changes during storage. I. Content of certain nutritive compounds Folia Horticulturae 6(1):41-51.

Selvakumar, G., Lenin, M., Thamizhiniyan, P. and Ravimycin, T. (2009). Response of Bioferilizers on the Growth and Yield of Blackgram (*Vigna Mungo* L) 1(4):169-175.

Setua, G.C., Das, N.K., Banerjee, N.D., Sengupta, T., Sudhakar, P., Sen, S. and Saratchandra, B. (2005). Effect of integrated nutrient management on quality leaf production in mulberry (*Morus alba*) under rainfed, alluvial soil conditions. Indian Journal of Agricultural Science 75:474-478.

Shah, H.C. (1959). Influence of magnesium nutrition on oil content of sunflower. Soil Science 87:320-324.

Sinclair, T.R. and Horie, T. (1989). Crop physiology and metabolism. Crop Sci. 29 : 90-98.

Stamp, N.E. (2003). Out of the quagmire of plant defense hypotheses Q. ReV. Biol 78:23-55.

Steward (1991). Plant Physiology Vol.10, Growth and Development, Academic Press, New York, pp. 359.

Tuncay, O., Esiyok, D., Yagmur, B. and Okur, B. (2011). Yield and quality of garden cress affected by different nitrogen sources and growing period. African Journal of Agricultural Research 6(3):608-617.

Wu FeiBo, Wu Liang Huan, Xu FuHua (1998). Chlorophyll meter to predict nitrogen sidedress requirements for short-season cotton (*Gossypium hirsutum* L.). Field Crops Research 56:309-314

Zhao, X., Carey, E.E., JE, Y., Wang and W., Iwamoto (2007). Influence of organic fertilization, high tunnel environment, and post harvest storage on phenolic compounds in lettuce Horticulture Science 42:71-76.

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Table 4. Biochemical contents in seeds of mungbean (PDM-54) during experimental field trials of 2004 and 2005

Treatments	Soluble sugar (g/100g)		Amino acid (g/100g)		Protein(g/100g)		Phenol(μg/g)		Ascorbic acid (mg/100g)		DNA(mg/100g)		RNA (mg/100g)	
	2004	**2005**	**2004**	**2005**	**2004**	**2005**	**2004**	**2005**	**2004**	**2005**	**2004**	**2005**	**2004**	**2005**
T$_1$	6.74^{d}	6.53^{bcd}	25.30^{e}	30.32^{e}	30.32^{cde}	28.65^{cd}	889^{e}	663^{e}	8.75^{de}	6.93^{abcd}	480.62^{abcde}	398.69^{de}	876.43^{abcde}	788.43^{cde}
T$_2$	9.76^{a}	6.71^{bcd}	44.16^{a}	34.52^{d}	37.00^{a}	28.93^{cd}	946^{a}	723^{d}	10.86^{a}	7.26^{abcd}	486.94^{a}	401.11^{d}	887.66^{ab}	810.77^{cd}
T$_3$	7.61^{bc}	7.53^{ab}	39.76^{bc}	40.12^{ab}	32.50^{bc}	33.31^{ab}	916^{c}	763^{b}	9.77^{bc}	8.13^{ab}	483.77^{abc}	489.36^{ab}	885.57^{abc}	854.56^{ab}
T$_4$	7.61^{bc}	7.25^{abc}	33.21^{d}	36.32^{c}	30.50^{cd}	31.54^{abc}	910^{cd}	748^{c}	9.31^{cd}	7.55^{abc}	480.76^{abcd}	411.37^{c}	885.41^{abcd}	827.09^{abc}
T$_5$	5.03^{f}	8.71^{a}	13.73^{e}	40.32^{a}	29.29^{f}	34.21^{a}	857^{f}	892^{a}	8.13^{e}	8.26^{a}	478.11^{abcde}	495.23^{a}	872.63^{abcdef}	881.78^{a}
T$_6$	8.42^{ab}		40.30^{b}		33.10^{b}		923^{b}		10.46^{ab}		486.64^{ab}		886.32^{ab}	

Note: Means followed by the same letter within a treatment were not significantly different at 5% level using Duncan's multiple range test (DMRT). Means of three replicates were taken.