
Biochemical changes in leaves of mustard under the influence of different fertilizers and cycocel

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Banerjee, A., Datta, J.K. and Mondal, N.K. (2012) Biochemical changes in leaves of mustard under the influence of different fertilizers and cycocel. *Journal of Agricultural Technology* 8(4): 1397-1411.

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 mustard by employing different varieties and different treatments such as different combined doses of biofertilizer and chemical fertilizer, different doses of growth retardant cycocel, different doses of compost. Untreated seeds were used as control. Among all the varieties, B₉ showed higher accumulation of chlorophyll, sugar, amino acid, ascorbic acid, phenol and proline. Treatment T₃ (3/4th chemical fertilizer :1/4th biofertilizer) and 300 ppm cycocel treatment stimulated the enhanced level of chlorophyll, sugar, ascorbic acid, phenol and proline in leaves of mustard. Higher accumulation of chlorophyll, sugar, amino acid, ascorbic acid, phenol and proline were recorded from T₄ (7.5 mt.ha⁻¹) treatment of compost. Combined application of biofertilizers, growth retardant and compost can therefore be employed for regulating crop metabolism and physiological responses resulting into better crop growth and enhanced protection against pathogens and pest.

Key words: Biochemistry, Chlorophyll, Phenol, Protein

Introduction

Oil seed groups being next to food crops hold sizable share of the countries gross cropped area (13%) India is the third largest producer of oil seeds in the world. It accounts for 19% of the world's area and 9% of the global production. (Sinha, 2003). Mustard is a major rabi crop requires relatively cool temperature, moist growing season and dry harvest period. 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

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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). 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) proposed 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, important group of bioactive compounds is that of phenolic compounds. Concerning the influence of fertilizer treatment on plant nutrient levels, compost treatments have stimulated sesame plant growth and have enhanced its pigment, carbohydrate, and mineral contents. 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).

In recent years a new class of organic chemicals has appeared with the special characteristics that they can retard or defer growth processes in plants, and those were termed “growth retarding chemicals” or “growth retardants” by Cathey (1964). According to him growth retardants are chemicals which suppress the overall growth and metabolism of plants by slowing down cell division and cell elongation without altering the gross morphology.

Form of nitrogen fertilizer could have an important influence on the accumulation of chlorophyll in plants. Lettuce plants fertilized with two forms of nitrogen ($\text{NO}_3 + \text{NH}_4$) 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 (Michalek and Rukasz, 1998). Nitrogen fertilization stimulates accumulation of chlorophyll. (Mihailovic *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. The higher total chlorophyll concentration of marigold leaves was observed when compost or vermicompost were added to the commercial horticultural plant growth medium than in pure medium (Atiyeh *et al.*, 2000). 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 defense 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 (Michalek and Rukasz, 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 NO₃. The same effect was observed in corn salad leaves in the case of second growing period. Of the organic constituents measured in plant tissue, ascorbic acid has frequently been reported on average to be higher in organically grown crops than with plants grown conventionally (Chen, 2005). Quality of lettuce crop was better when treated with vermicompost and compost than with mineral fertilization (higher vitamin C concentration and lower nitrate accumulation) (Premuzic *et al.*, 2002). This study, therefore, was conducted to study the effects of different fertilizers and growth retardant cycocel on the levels of macromolecules in leaves of mustard (*Brassica campestris* cv.B₉) under field condition.

Material and methods

Field experiments for the experiment of *Brassica campestris* cv.B₉ were conducted during winter season of 2005-2006, 2006- 2007 and 2007-2008 at the agricultural farm of Burdwan University, India at latitude 87°50'51"E and longitude 23°15'12"N). During the winter season (2006), we conducted two field experiments. In the first experiment, six different combinations of biofertilizer and chemical fertilizer were applied in field as separate treatments, including T₁: recommended dose of chemical fertilizer (N:P:K-100 : 50 : 50), T₂: 1/2 chemical fertilizer:1/2 biofertilizer, T₃: 3/4th chemical fertilizer:1/4th biofertilizer, T₄: 3/4th biofertilizer:1/4th chemical fertilizer, T₅: recommended dose of biofertilizer and T₆: control-without any chemical fertilizers. In the second experiment, mustard seeds were soaked overnight in the solution of growth retardant and cycocel at six different levels viz., CCC-100 ppm, CCC-200 ppm, CCC-300 ppm, CCC-400 ppm, CCC-500 ppm, and control (without any growth retardant), were sown in the field 24 h after soaking. In 2007, six different levels of compost (control-without any compost, T₂-4.5 mt.ha⁻¹, T₃-6.0 mt.ha⁻¹, T₄- 7.5 mt.ha⁻¹, T₅- 9.0 mt.ha⁻¹, T₆- 10.5 mt.ha⁻¹) were used along with the best screened dose of combined doses of biofertilizer, chemical fertilizer, and growth retardant cycocel in 2006.

The experiments were conducted in randomized block design with three replications within individual plot sizes of 5 m×5 m, row to row spacing of 50 cm and plant to plant spacing of 15 cm. The land was mechanically ploughed and harrowed during land preparation after plot setting. Irrigation channels in 0.5 m wide were in between the replications to ensure the easy and uninterrupted irrigation of the individual plots independently until crops grew up to the maturity stage.

Physiologically active leaf (3rd and 4th from the top) samples from ten different plants of each plot were plucked randomly for determination of different biochemical parameters.

Composite samples of leaf were crushed separately in a mortar with 10ml of distilled water, poured in test tube and centrifuged at 10000 rpm for ten minutes. The supernatant was used for soluble sugar estimation by using anthrone reagent as described by Mc Cready *et al.* (1950). Free amino acid in leaf samples were determined by using the Moore and Stein (1948) method. Chlorophyll content (mg g⁻¹ fresh weight) was determined through organic solvent (80% acetone) extraction method as described by Arnon (1949). Protein content (mg 100 g⁻¹) in leaf samples were determined by using the method of Lowry *et al.*, (1951). Ascorbic acid content (mg 100 g⁻¹) in leaves were determined by using trichloroacetic acid extraction method as described by Mukherjee and Chaudhuri (1983). Proline content in leaves were determined by following methods of Bates *et al.* (1973). Phenol content in leaves were determined by following methods of Malick and Singh (1980).

Data were computed by analysis of variance (ANOVA), with treatments as the independent variable. All statistical analysis was carried out with the program SPSS 11.0 for windows. All values were expressed as mean values. The statistical significance of differences between the different treatments were compared using Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$.

Results

This study pertained to assess the biochemical changes in leaves of mustard under different treatments of biofertilizer, chemical fertilizer, compost and growth retardant cycocel and was evaluated in terms of chlorophyll, protein, amino acid, sugar, ascorbic acid, phenol and proline content to enhance crop growth, metabolism and optimum protection against pathogens and pest.

In 2005-2006, there was significant level of variation in the level of total chlorophyll in the leaves of seven mustard varieties. Minimum and maximum total chlorophyll levels were observed in V₇ and V₃ respectively (Table 1). All the combined treatments of biofertilizer and chemical fertilizer significantly

increased the level of total chlorophyll upto T₃ treatment (3/4th biofertilizer: 1/4th chemical fertilizer) but decreased in T₄ and T₅ treatment (Table 2). It was observed that the total chlorophyll level significantly increased in all the treatments of biofertilizer and chemical fertilizer with respect to control (T₆). Application of growth retardant cycocel promoted stimulatory effect upto 300 ppm and then showed inhibitory effect at 400ppm and 500ppm concentrations for the levels of total chlorophyll in leaves. The level of total chlorophyll contents significantly increased in all the cycocel treatments in comparison to control (Table 3). In 2007-2008, application of compost significantly increased the level of total chlorophyll up to 7.5 mt. ha⁻¹ and then decreased at further higher concentrations. The levels of total chlorophyll in leaves were found to be higher in all the compost treatments in comparison to control without any compost treatment (Table 4).

The minimum and maximum total soluble sugar contents were observed in V₇ and V₃ respectively followed by other studied varieties (Table 1). Application of biofertilizer along with chemical fertilizer significantly increased the level of total soluble sugar in leaves of crop plants in comparison to the plots without application of any form of fertilizer (Table 2). The cycocel application was found to be stimulatory upto 300ppm and then reduced at further higher concentration. The level of total soluble sugar content in leaves were found to be higher in all the cycocel treated plots when compared to control (Table 3). Similar results in relation to sugar content were observed in mulberry leaves (Setua *et al.*, 2005). Level of total soluble sugar content in leaves increased significantly through compost application when compared to control (Table 4).

The different studied varieties showed differential response towards accumulation of amino acid content in leaves. Maximum accumulation were observed in case of variety V₃.(Table 1) Impact of application of biofertilizer along with chemical fertilizer were found to be inhibitive in terms of total free amino acid content in leaves when compared to control (Table 2). Application of cycocel and compost significantly increased the level of total free amino acid in leaves when compared to control (Table 3 and 4).

Total protein content in leaves varied significantly among the different studied varieties. The lowest and highest value was observed in V₆, V₇ and V₅. (Table 1) Application of biofertilizer and cycocel significantly promoted considerable variation among the different treatments. The level of total protein content increased upto T₂ treatment (½ chemical fertilizer: ½ biofertilizer and 200 ppm cycocel) and then reduced significantly (Table 2 and 3). In 2007-2008, application of compost significantly promoted the level of total protein content in leaves when compared to control (Table 4).

Level of ascorbic acid in leaves showed significant level of variation among the seven mustard varieties (Table 1). Application of biofertilizer significantly affected the level of ascorbic acid content in leaves of crop plants. There was a steady decrease in the level of ascorbic acid in leaves upto T₄ treatment (1/4th Chemical fertilizer: 3/4th biofertilizer) and then increased significantly at T₅ (recommended dose of biofertilizer). The level of ascorbic acid content in leaves increased in all the biofertilizer treated plots when compared to control (Table 2). Application of cycocel significantly increased upto 300ppm and then reduced significantly at further higher concentration. The level of ascorbic acid in leaves increased in all the cycocel treated crop plants when compared to control (Table 3). The effect of compost were found to be stimulatory upto 7.5 mt.ha⁻¹ and then reduced significantly at further higher concentration (Table 4).

The maximum accumulation of phenol in leaves were observed in V₃ (Table 1). Enhanced level of phenol content in leaves of biofertilizer and cycocel treated plots were recorded with respect to control (Table 2 and 3). Application of compost was found to be stimulatory for phenol accumulation in leaves of mustard plant. Higher level of phenolic compounds frequently have been reported in organically grown crops than in conventionally grown crops.(Carbonaro *et al.*, 2002; Lombardi Boccia *et al.*, 2004; Sousa *et al.*, 2005).

In 2005-2006, there is significant level of variation in the proline content of leaves of seven varieties of mustard (Table 1). Biofertilizer treatments in combination with chemical fertilizers showed differential biochemical response in terms of osmotic balance of the crop plants resulting into significant variation in the level of proline in leaves (Table 2). Significant level of proline accumulation in leaves of cycocel treated crop plants were observed during the present investigation (Table 3). The different graded dose of compost along with application of biofertilizer, chemical fertilizer and growth retardant treated plots promoted elevated level of proline in the leaves of crop plants (Table 4).

Table 1. Biochemical contents of leaves of seven mustard varieties during experimental field trial of 2005-06

Variety	Total Chlorophyll (mgg ⁻¹ fw)	Total Soluble Sugar (mgg ⁻¹)	Total free Amino Acid (mgg ⁻¹)	Total Protein (mgg ⁻¹)	Total ascorbic acid (mgg ⁻¹)	Total phenol (mgg ⁻¹)	Total proline (mgg ⁻¹)
V ₁	0.577 ^{bcd}	9.079 ^{ef}	0.876 ^c	60.53 ^{bcd}	0.132 ^{abcdef}	0.031 ^{abcdef}	0.134 ^{abcdef}
V ₂	0.736 ^{bcd}	12.621 ^{abcd}	1.125 ^c	69.334 ^{bcd}	0.173 ^{abc}	0.035 ^{abcd}	0.427 ^{abcd}
V ₃	1.435 ^a	20.658 ^a	2.770 ^a	73.956 ^{bc}	0.196 ^a	0.039 ^a	0.698 ^a
V ₄	0.770 ^b	13.148 ^{ab}	0.846 ^f	75.937 ^b	0.182 ^{ab}	0.038 ^{ab}	0.693 ^{ab}
V ₅	0.657 ^{bcd}	9.752 ^e	0.531 ^e	89.144 ^a	0.137 ^{abcd}	0.034 ^{abcde}	0.332 ^{abcde}
V ₆	0.756 ^{bc}	12.972 ^{abc}	0.976 ^d	58.109 ^f	0.148 ^{abcd}	0.036 ^{abc}	0.428 ^{abc}
V ₇	0.426 ^{bcd}	8.811 ^{ef}	2.553 ^{ab}	58.109 ^f	0.121 ^{abcdef}	0.029 ^{abcdef}	0.038 ^{abcdef}

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 2. Biochemical contents of leaves of mustard variety (B₉) during first experimental field trial of 2006-07

Treatments	Total Chlorophyll (mgg ⁻¹ fw)	Total Soluble Sugar (mgg ⁻¹)	Total free Amino Acid (mgg ⁻¹)	Total Protein (mgg ⁻¹)	Total ascorbic acid (mgg ⁻¹)	Total phenol (mgg ⁻¹)	Total proline (mgg ⁻¹)
T ₁	0.237 ^{abcd}	8.415 ^{bcd}	17.250 ^{ab}	66.91 ^b	1.259 ^c	0.034 ^{cd}	0.498 ^{bc}
T ₂	0.416 ^{ab}	8.751 ^b	13.936 ^{de}	99.929 ^a	0.699 ^{de}	0.039 ^b	0.543 ^b
T ₃	0.443 ^a	11.237 ^a	16.086 ^c	25.532 ^c	0.740 ^d	0.049 ^a	1.126 ^a
T ₄	0.331 ^{abc}	8.728 ^{bcd}	15.256 ^d	10.081 ^e	0.642 ^{de}	0.037 ^c	0.474 ^{bcd}
T ₅	0.229 ^{abcde}	8.304 ^{bcd}	14.968 ^{de}	62.071 ^{bc}	1.421 ^b	0.029 ^c	0.427 ^{bcd}
T ₆	0.182 ^{abcde}	7.732 ^{bcd}	17.731 ^a	60.309 ^{bcd}	1.459 ^a	0.017 ^f	0.421 ^{bcd}

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 of leaves of mustard variety (B₉) during second experimental field trial of 2006-07

Treatments	Total Chlorophyll (mgg ⁻¹ fw)	Total Soluble Sugar (mgg ⁻¹)	Total free Amino Acid (mgg ⁻¹)	Total Protein (mgg ⁻¹)	Total ascorbic acid (mgg ⁻¹)	Total phenol (mgg ⁻¹)	Total proline (mgg ⁻¹)
T ₁	0.521 ^{bc}	28.030 ^{abc}	13.199 ^{abc}	95.087 ^{ab}	0.685 ^{bcd}	0.038 ^c	0.611 ^c
T ₂	0.619 ^b	30.355 ^{ab}	8.664 ^{abcde}	107.853 ^a	1.350 ^b	0.041 ^b	0.733 ^b
T ₃	1.229 ^a	30.540 ^a	15.225 ^{ab}	65.692 ^{cd}	2.066 ^a	0.047 ^a	1.054 ^a
T ₄	0.482 ^d	25.481 ^d	12.532 ^{abcd}	25.973 ^c	1.293 ^{bc}	0.025 ^d	0.504 ^d
T ₅	0.367 ^{de}	22.162 ^e	16.024 ^a	73.076 ^c	1.144 ^{bcd}	0.023 ^e	0.429 ^e
T ₆	0.330 ^{de}	21.276 ^e	10.203 ^{abcde}	31.696 ^c	0.545 ^{bcd}	0.019 ^f	0.163 ^f

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 4. Biochemical contents of leaves of mustard variety (B₉) during experimental field trial of 2007-08

Treatments	Total Chlorophyll (m ^g g ⁻¹ fw)	Total Soluble Sugar (m ^g g ⁻¹)	Total free Amino Acid (m ^g g ⁻¹)	Total Protein (m ^g g ⁻¹)	Total ascorbic acid (m ^g g ⁻¹)	Total phenol (m ^g g ⁻¹)	Total proline (m ^g g ⁻¹)
T ₁	0.515 ^{bcd}	13.092 ^{abcde}	0.508 ^{de}	32.135 ^{abcde}	0.223 ^{cde}	0.028 ^{abcde}	5.237 ^f
T ₂	0.818 ^{bc}	16.497 ^{abc}	1.086 ^d	47.543 ^a	0.246 ^c	0.034 ^a	10.411 ^c
T ₃	0.828 ^b	18.065 ^{ab}	0.314 ^f	39.179 ^{ab}	0.377 ^{ab}	0.034 ^{ab}	10.711 ^b
T ₄	1.394 ^a	19.117 ^a	3.826 ^a	17.101 ^f	0.411 ^a	0.034 ^{abc}	11.213 ^a
T ₅	0.759 ^{bcd}	16.417 ^{abcd}	2.281 ^b	35.877 ^{abc}	0.239 ^{cd}	0.030 ^{abcd}	10.048 ^d
T ₆	0.758 ^{bcd}	14.937 ^{abcde}	1.462 ^c	33.676 ^{abcd}	0.226 ^{cde}	0.029 ^{abcde}	6.926 ^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.

Discussion

The significant variation in the level of total chlorophyll content in physiologically active leaves of seven mustard varieties may be due to variable rate of biosynthesis of chlorophyll and photosynthesis depending upon the genetic potential of the mustard varieties. Significant enhancement in total chlorophyll content in leaves of all the treatments of biofertilizer and chemical fertilizer with respect to control (T₆) due to increased uptake of magnesium from the soil in the form of Mg⁺² under the influence of biofertilizer application and also the beneficial effects of bacterial inoculation on increased chlorophyll content due to higher availability of nitrogen to the growing tissue and organs supplied by nitrogen fixing *Azotobacter* species. Our results also confirm the earlier findings of Dev choudhary and Bajaj (1980); Chandrasekhar *et al.* (2005). The total chlorophyll content in leaves of mustard under cycocel treatment was found to be stimulatory with respect to control which may be due to the stimulating effect of cycocel towards intensifying the green colour of foliage (Cathey, 1975). The increased level of total chlorophyll concentration in leaves of all the cycocel treated plots might be due to the influence of growth retardant on delaying leaf senescence and hence keeping the green pigment from degradation (Wafsy and El-Din, 1995). The results are in agreement with some earlier works (Hosni, 1996; Yoon and Lang, 1998; Mostafa, 2000).

Compost application was found to be effective towards promoting enhancement in total chlorophyll content in leaves of mustard due to combined application of biofertilizer, compost and growth retardant. This combined application might have stimulated the rate of chlorophyll biosynthesis with adequate supply of nitrogen under the influence of compost, growth retardant and biofertilizer application. The elevated level of chlorophyll in leaves of crop

plants under different treatments of biofertilizer, chemical fertilizer, compost and growth retardant are of specific importance as photosynthetic activity of crop plants and crop yield increases with elevated level of chlorophyll content in leaves (William *et al.*, 1990). Our findings corroborates with earlier findings (Mona, 2006).

Significant level of variation in total soluble sugar content in leaves among the different varieties may be attributed towards the variable rate of photosynthesis leading to production of variable amount of photosynthate among the different varieties. Higher biosynthesis of chlorophyll and photosynthesis of flag leaf of plants under the biofertilizer treated plots might have resulted towards higher level of total soluble sugar content of leaves. The results also reveal that dual inoculation of biofertilizers have pronounced influence on biosynthesis of carbohydrates in leaves (Ram Rao *et al.*, 2007). Higher accumulation of sugar in leaves of mustard under cycocel treated plots might be due to higher rate of photosynthesis. Our results also confirm the earlier findings Uprety and Yadava (1985) in oat plants. During the field trial of 2007-08, the overall reduction of total soluble sugar content in leaves of all the treatments with respect to the previous field trials of 2005-2006 and 2006-2007 may be attributed towards redistribution of sugar from the vegetative part to the reproductive part of the plant. This is probably due to transportation of soluble sugar from the flowering parts are used by the developing seeds in the crop plants. Improved level of sugar in the leaves of crop plants under compost treatments can be attributed towards the combined application of biofertilizer, compost and growth retardant cycocel which may have stimulated the rate of photosynthesis leading to higher rate of production of photosynthate in the leaves along with adequate supply of nutrients. Our findings were similar with the earlier findings on *Siderites montana* (El-Sherbeny *et al.*, 2005) and on *Iris* (Ali, 2005). These carbohydrate changes are of particular importance because of their direct relationship with such physiological processes as photosynthesis, translocation and respiration.

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 transport 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, defense and reproductive processes. Application of combined doses of biofertilizer and chemical fertilizer was found to be inhibitory towards free amino acid content in leaves which might be attributed towards lesser uptake of nitrogen from soil by the crop plants under treated plot. Application of cycocel was found to be stimulatory towards higher biosynthesis and accumulation of

amino acid in leaves of mustard. The enhanced level of free amino acids in leaves of mustard under compost treated plots might be due to effective conversion of nitrogen content amino acids to free amino acids under ready variability of mineral elements in the soil and their translocation in the plant system in presence of combined application of biofertilizer and compost (Ram Rao *et al.*, 2007).

Significant level of variation in the level of total soluble protein content in leaves of different varieties of mustard under the field trial of 2005-2006 reveals differential rate of nitrogen assimilation by crop plants. The progressive increase in the leaf protein content up to T₂ treatment of combined dose of biofertilizer and chemical fertilizer may be due to higher absorption of nitrogen and phosphorous from soil by crop plants due to their availability under the influence of application of biofertilizer (Chela *et al.*, 1999; Uyanoz, 2007). The protein content in leaves was found to be higher in all the cycocel treated plants with respect to control. It can be inferred from the earlier reports (Glenn *et al.*, 1980; Yokoyamah, 1984; Rittig, 1987) that the increase in the level of total soluble protein content in the leaves of cycocel treated plants can be explained in terms of retarded growth (smaller plants with more concentrated cell nitrogen content) and delayed senescence with longer retention of nitrogen in the leaves before translocation into generative organs. Significant variation in the level of protein content in leaves of mustard plants under compost treated plots reflects the variable rate of nutrient uptake by the crop plants.

Ascorbic acid content in leaves showed significant variation among the different mustard varieties due to the influence of different factors including cultivar, nitrogen rate, environmental factors (especially light), stage of growth and storage (Dumus *et al.*, 2003). The higher level of ascorbic acid content in leaves of mustard plants under biofertilizer treated plots might be attributed towards higher concentration of sugar which is essential for rapid biosynthesis of ascorbic acid (Chinoy *et al.*, 1961). The declining trend in the level of ascorbic acid in leaves from treatment T₁ onwards up to T₄ which might be attributed towards biofertilizer application leading to higher availability of nitrogen to crop plants which increased the vegetative growth of crop plants and caused more shading of fruit, thereby lowering the ascorbic acid content. (Dumus *et al.*, 2003) The application of growth retardant may have stimulated the biosynthesis of sucrose and other sugars which is essential for rapid biosynthesis of ascorbic acid and thus resulted into enhanced level of ascorbic acid in cycocel treated plants (Chinoy *et al.*, 1961). Application of compost was found to be inhibitory in terms of accumulation of ascorbic acid in leaves of mustard plants due to higher availability of nitrogen and reducing the level of ascorbic acid (Montagu and Goh, 1990; Lester, 2006).

Phenol content in leaves of mustard plants significantly varied among the studied mustard varieties reflecting differential response of studied mustard varieties towards insect attack, resulting in variable amount of total phenol accumulation in leaves. Our findings also confirm the earlier findings of Zhao *et al.*, (2007) where phenolic compounds in lettuce were not consistently affected by nutrient source but numerous factors including growing environment, season and cultivar, affected phenolic compounds with cultivar differences being most significant. Higher phenol content in leaves of biofertilizer treated plots might be attributed towards the use of accumulated nitrate in plant thus enabling it to use more carbohydrates for structural growth under the influence of biofertilizer application (Hanafy Ahmed *et al.*, 2000). Antigerberellic nature of growth retardant cycocel may have promoted enhanced level of total phenolics in leaves of mustard cycocel treatment. Elevated level total phenol content in leaves of mustard in the compost treated plots in comparison to control may be attributed towards the organic manure compounds (Young *et al.*, 2005).

Proline content in leaves of the different studied varieties of mustard reflects the differential response of the seven cultivated mustard varieties towards environmental stress and different level of osmotic adjustment (Ozturk and Demir, 2002). Significant variation in the level of accumulation of proline in leaves of mustard under different treatments of biofertilizer and chemical fertilizer might be attributed towards the response of the biofertilizer treated crop plants towards mitigative and stimulating effect of drought tolerance (Sheteawi and Tawfik, 2007). Application of growth retardant cycocel stimulated the accumulation of proline in leaves of mustard and therefore reflects the osmotic adjustment of crop plants. Osmoregulation has been known to be associated with maintenance of leaf area water extraction due to root growth, continued photosynthesis and diversion of assimilates towards sink (Wright *et al.*, 1983). For regulating the osmotic balance of crop plants synthesis and accumulation of secondary metabolites such as proline in leaves of mustard were recorded under different treatment of compost. Our findings support the earlier findings of Sheteawi and Tawfik (2007).

This study concludes that combined application of biofertilizers, growth retardant and compost reflects the variable biochemical response of mustard plants towards synthesis and accumulation of simple organic molecules such as sugar, free amino acid, chlorophyll and proline in leaves which therefore played role in regulation of plant osmosis and crop plant metabolism leading to better crop growth and yield. Combined application of biofertilizer, compost and growth regulator cycocel stimulated the accumulation of certain metabolites for optimum plant growth along with enhanced plant defense system against insect

and disease attack as well as through antioxidant defense mechanism which may adversely effect the growth and yield attributes of crop plants under agroclimatic condition of old alluvial soil, Burdwan, India. While the results are interesting in their own right, the major impact of the work may be that there is the potential for combine application of biofertilizers, growth retardant and compost to increase the protein, sugar and ascorbic acid content of other crops, perhaps forages for animals and food crops for human consumption, without a sacrifice of yield. Nonetheless, more research efforts and participatory evaluation of the combined application of different forms of fertilizer and growth retardant cycocel are needed in this area.

Acknowledgements

This work was supported by University Grants Commission (UGC) under UGC major research project having basic research grant from Government of India (RefNo. 30-109 / 2004 (SR)dt. 10.11.2004). Author is highly grateful to Prof. J.K. Datta, the Principal Investigator of this project and to all staff members of Crop Research and Seed Multiplication Farm of Burdwan University, West Bengal, India for carrying out the field experiments for subsequent three years.

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(Published in July 2012)