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FULL LENGTH ARTICLE

Chemical fertilizer in conjunction with biofertilizer and vermicompost induced changes in morpho-physiological and bio-chemical traits of mustard crop

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Abstract To study the impact of reduced dose of chemical fertilizer and its combination with biofertilizer and vermicompost on morpho-physiological and biochemical traits of mustard (*Brassica campestris* cv. B_9), field experiments were conducted during winter seasons of November to February 2011–2012 and 2012–2013 respectively in an old alluvial soil zone of Crop Research and Seed Multiplication Farm, Burdwan University, Burdwan, West Bengal, India. Mustard was cultivated using a full recommended dose of chemical fertilizer (N:P:K–100:50:50) and along with six different reduced doses of chemical fertilizer combined with biofertilizers and vermicompost. The performance of the crop was adjudged in terms of various parameters viz. leaf area index (LAI), leaf area duration (LAD), leaf area ratio (LAR), crop growth rate (CGR), net assimilation rate (NAR), photosynthetic rate (PR), harvest index (HI) and biochemical attributes such as total chlorophyll, sugar and proline content of physiologically active leaves of mustard. Differential significant ($p < 0.05$) treatment response was reflected for the studied traits during crop maturity. The data revealed that vermicompost application significantly stimulated most of the studied attributes. It was concluded that 25% reduced dose of chemical fertilizer and

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its combination with vermicompost (T4) was optimum for most of the parameters studied as compared to the control at both crop stages.

1. Introduction

India is the third largest producer of oil seeds in the world. It accounts for 19% of world's area and 9% of the global production (Sinha, 2003). Mustard (*Brassica campestris*) is an important oil seed crop, next to sunflower. Application of chemical fertilizers has contributed significantly to the huge increase in the world food production. But the adverse impacts of excessive inputs of chemical fertilizers in conventional agricultural practices are being well documented (Banerjee et al., 2011; Garai et al., 2014). Chemical fertilizers also have contributed significantly toward the pollution of water, air and soil. In agro-ecosystems, the use of synthetic toxic chemical pesticides affects the soil fertility and growth of cultivated crops (Ignacimuthu and Vendan, 2007). At present we are using chemical fertilizers in great quantities to compensate the deficiency of nutrients in soil. For minimizing the accumulation of pollutants in agro-ecosystems we should avoid the use of toxic chemicals especially synthetic chemical pesticides and fertilizers in agricultural process. Organic products are eco-friendly natural sources, which can be considered as an alternative to sustainable agriculture development. In India, as a step toward the expansion of native sources, the application of organic waste materials will be useful for achieving higher production. The impact of organic agriculture on natural resources favors interactions within the agro-ecosystem that is vital for both agricultural production and nature conservation. The current trend is to explore the possibility of supplementing chemical fertilizers with organic ones that are eco-friendly and cost-effective.

In the above context, eco-friendly and environmentally safe biofertilizer, vermicompost, etc. became handy to minimize chemical fertilizer use as well as a carbon sink in such crop fields. In recent years, vermicomposting has turned out to be promising way out for safe disposal of organic waste. It is a technique of biodegradation or stabilization of organic waste (natural/anthropogenic) by using earthworms and microbes (Garg et al., 2006; Suthar, 2007; Mainoo et al., 2009). Earthworm plays a major role in plant material degradation and this concept is used in vermicomposting technology with the supplement of cow dung source to enhance plant growth. Cow dung is an organic and nitrogen rich material, and it can be easily degraded in the soil. Cow dung also exhibits plant growth promoter properties (Nattudurai et al., 2014).

The use of biological nitrogen fixation by living nitrogen fixers will help to minimize use of chemical nitrogen fertilizer and to improve plant growth to decrease the production cost and environmental risk (El-Hawary et al., 2002). Bacterial fertilization of non-legume crops by nitrogen fixing bacteria has assumed great importance in recent years. In our present investigation, phosphate solubilizing bacteria (PSB) and *Azotobacter* are used as biofertilizer. PSB secrete some organic acids which can solubilize P from insoluble and fixed forms to plant available forms, whereas *Azotobacter* can convert atmospheric N_2 into plant available form of N in the soil. In the recent years, among the various sources of organic manure, efficacy of vermicompost was reported manifold. Earthworms consume large quantities of organic matter and excrete it as cast and this cast contains several enzymes and is rich in plant nutrients, which are beneficial for bacteria and mycorrhizae (Reddy and Reddi, 2002). They also noted that vermicompost is an excellent base for the establishment of beneficial non-symbiotic and symbiotic microbes. Different levels of nitrogen significantly influenced LAI, total dry matter accumulation, CGR, but plant height was not significantly influenced by the different levels of nitrogen application. On the other hand, 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.

This study, therefore, was conducted to evaluate the effects of integrated nutrient management (chemical fertilizers, biofertilizer and vermicompost) on the morph-physiology and macromolecular changes in leaves of mustard (*B. campestris* cv. B_9) and to screen out the best fertilizer combination under this field condition.

2. Materials and methods

2.1. Experimental site

Field experiments were conducted at the Crop Research and Seed Multiplication Farm, Burdwan University, Burdwan, West Bengal, India. The latitude is 87°50′37.35″E and longitude is 23°15′7.29″N with an average altitude of 30 m above the mean sea level during the winter seasons of 2011–2012 and 2012–2013 with rapeseed (*B. campestris* L. cv. B9).

2.2. Climatic condition

Weekly minimum and maximum temperature, total rainfall, sunshine hours, wind speed, and relative humidity (RH) were recorded. Some climate factors were collected and analyzed. Climate factors in both growing periods were similar but not the same. Both the growth periods started with moderate temperature where the maximum temperature was around 29 °C and the minimum temperature was around 16 °C, and then they cooled and ended again with similar maximum and minimum temperatures. The mean temperature and high relative humidity (ranging between 75% and 90%) were similar for both growing cycles. The mean wind speed (1.3–8.1 km h^{-1}) and mean sunshine (4.23–7.15 h day^{-1}) were almost the same in both growing seasons. No rainfall was recorded in the first growing season (2011–2012), but in the second season 0–5 mm rainfall was recorded on average.

2.3. Treatment combinations and design of experimental field

The treatments used were as follows:

T1 – Full recommended dose of chemical fertilizers (100:50:50, that is 100 kg ha^{-1} N:50 kg ha^{-1} P:50 kg ha^{-1} K).
T2 – 50% of the recommended dose of chemical fertilizers (50 kg ha^{-1} N:25 kg ha^{-1} P:25 kg ha^{-1} K) + vermicompost (2.5 t ha^{-1}).
T3 – 50% of the recommended dose of chemical fertilizers (50 kg ha^{-1} N:25 kg ha^{-1} P:25 kg ha^{-1} K) + dried cow dung (2.5 t ha^{-1}).
T4 – NPK (75% of full dose) + vermicompost (2.5 t ha^{-1}).
T5 – NPK (75% of full dose) + dried cow dung (2.5 t ha^{-1}).
T6 – NPK (75% of full dose) + vermicompost (2.5 t ha^{-1}) + *Azotobacter* and PSB (7.5 kg ha^{-1} each).
T7 – NPK (75% of full dose) + dried cow dung (2.5 t ha^{-1}) + Azotobacter and PSB (7.5 kg ha^{-1} each).

The treatment combinations were replicated thrice and arranged in a randomized block design (RBD). Individual plot sizes were 4 m × 2.5 m. Row-to-row and plant-to-plant spacing was 30 cm and 15 cm, respectively. Irrigation channels measuring 0.5 m wide were between the replications to ensure easy and uninterrupted flow of irrigation for each individual plot. The same treatments were followed for two consecutive years. Chemical fertilizers were applied at the recommended NPK dose (100:50:50) for mustard as per the Directorate of Agriculture, Government of West Bengal (Bhattacharyya, 1998). Treatment T1 was the control. A biofertilizer containing *Azotobacter chrococcum* and phosphate solubilizing microorganism (*Bacillus polymyxa*) was applied at the rate of 7.5 kg ha^{-1}, each through seed inoculation. The biofertilizer was collected from Nitrofix Laboratories, 25, Bansdoroni Avenue, Kolkata-70. The colony-forming unit (cfu) of *Azotobacter* and PSB was 2.3 × 109 and 2.1 × 108 cells per gram of carrier material, respectively.

2.4. Pre-harvesting soil condition

Physical, chemical and biological characteristics of the initial soil (0–15 cm depth) were mechanical fractions such as sand (0.02–0.2 mm) (%) – 22.34, silt (0.002–0.02 mm) (%) – 38.35, clay (< 0.002 mm) (%) – 26.24, pH (1:2.5, soil: water) – 5.89, CEC (cmol kg^{-1}) – 10.21, Organic C (%) – 0.98, Available N (mg kg^{-1}) – 12.02, Available K (mg kg^{-1}) – 10.19, Available P (mg kg^{-1}) – 12.53, Total bacteria (cfu g^{-1} of soil) – 8.6×10^6, and Total Fungi (cfu g^{-1} of soil) – 1.2×10^3. All the detailed pre and post-harvesting soil characteristics of this experiment were previously discussed in our published article (Mondal et al., 2015).

2.5. Crop establishment

The seeds were soaked in distilled water for 24 h. The biofertilizer (0.1 kg) was mixed with seeds (1.0 kg) and after that the seeds were sown separately in (4 × 2.5 m^2) plots. Sowing dates were 6 December 2011 and 11 December 2012. The plant density was approximately 28–32 plants per m^2 land area. Dates of harvesting were 11 March 2012 and 20 March 2013. Chemical fertilizers were used in the form of urea, single super phosphate, and muriate of potash and it was added after layout preparation. Seed sowing was done after 10 days of application of chemical fertilizers. Vermicomposts and organic fertilizers were used after 5 days of seed sowing at a basal dose (2.5 t ha^{-1}), and a gap of 15 days was maintained between the application of organic fertilizers and chemical fertilizers. As per the guidelines of the Department of Agriculture, chemical fertilizers were used in two splits such as 1/2 N + full P + full K as basal and 1/2 N as top dressing. Two hand-weedings at 15–18 DAS (days after sowing) and 38–40 DAS were carried out. The crops of each plot were harvested separately when 90% of the plant with silique became golden yellow in color.

2.6. Vermicompost preparation and application

For the preparation of vermicomposts, a pit of 1.5 m × 2 m and 1.5 m deep was prepared. Then the pit was filled with cow dung collected from the surrounding villages and *Eichhornia* from local wetlands. Here, cow dung and dried chopped *Eichhornia* are used as composting materials. Cow dung and chopped dried *Eichhornia* are mixed in the proportion of 3:1 and are kept for partial decomposition for 15–20 days. No replication was done. Special types of earthworms such as *Eisenia fetida* (1500–2000 numbers per pit) were used for the production of vermicomposts. A final layer of soil was applied over the compost pit and allowed to remain for 3 months for bacterial decomposition to take place. The vermicomposting unit should be kept in a cool, moist, and shady site. Therefore, 18–25 °C temperature should be maintained for proper decomposition. The optimum moisture level (30–40%) was maintained. By regular sprinkling of water, the optimum moisture level was maintained. After 3 months, the compost was taken out from the pit and applied to the experimental field. The nutrient availability in vermicomposts was 1.71% N, 1.18% P, 0.98% K, 0.0088% Zn, 0.094% Fe, 0.024% Mn, and 0.012% Cu and that in cow dung was 0.98% N, 1.01% P, 0.54% K, 0.0056% Zn, 0.077% Fe, 0.016% Mn, and 0.009% Cu (Mondal et al., 2014). The first irrigation was applied after seed sowing, and afterward the crop was irrigated at regular intervals ranging from 15 days up to 55 days. The crop exhibited no sign of insect/pest attack and disease incidence. The experimental site was kept free of weeds by intercultural and hand hoeing. All the morphological and yield attributes and changes in soil physical and physicochemical properties of this experiment were previously discussed in our two previously published articles (Mondal et al., 2014, 2015) and here only pre- and post-harvesting soil characteristics are investigated and described.

2.7. Plant materials

Dry matter and Leaf area were sampled at an interval of 15 days from 30, 45 and 60 days of sowing to quantify effects of environmental influences on effect of different combined doses of chemical and bio-fertilizer with vermicompost and compost. Rate of dry matter accumulation varies across to the life cycle of a crop and physiologically active leaves. During these two consecutive years, influence of seven

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different combined doses of chemical, biofertilizers and vermicompost on morpho-physiological traits and biochemical attributes viz., LAI, LAD, LAR, CGR, NAR, PR, HI, total chlorophyll, sugar and proline of physiologically active leaves of mustard was studied and the field data were analyzed statistically.

2.8. Parameters studied

The observations on morpho-physiological traits, LAI, LAD, LAR, CGR, NAR, PR, HI were recorded at different stages, i.e., 30 days after sowing (DAS), 45 days after sowing (DAS), and 60 days after sowing (DAS) of crop growth. Leaf area was measured with an electronic leaf area meter (LAM 101, Disha Online Pvt. Ltd., Raipur, Chattisgarh, India).

The leaf index was calculated using the formula (1) given by Watson (1947)

$$\mathrm{LAI} = \frac{Leaf\ Area}{Land\ Area} \quad (1)$$

Leaf area ratio is the ratio of leaf area and total biomass. It was calculated with the following formula (2):

$$\mathrm{LAR} = \frac{\mathrm{LA}}{W}\ \mathrm{m^2\ g^{-1}} \quad (2)$$

where LA = leaf area in m^2 and W = dry weight of plant.

Leaf area duration (LAD) was calculated using the following formula (3):

$$\mathrm{LAD} = \frac{(\mathrm{LAI}_1 + \mathrm{LAI}_2) \times (t_2 - t_1)}{2} \quad (3)$$

where LAI_1 and LAI_2 are leaf area indices recorded at times t_1 and t_2, respectively.

The dry weight per plant was calculated and used to estimate crop growth rate (CGR) as proposed by Hunt (1978) (4):

$$\mathrm{CGR} = \frac{W_2 - W_1}{t_2 - t_1}\ \mathrm{g\ m^{-2} day^{-1}} \quad (4)$$

where W_1 and W_2 are dry weights (g m^{-2}) at first and second harvests taken at times t_1 and t_2 respectively.

Net assimilation rate (NAR) was calculated by using the formula (5) given by Watson (1952):

$$\mathrm{NAR} = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{L_2 - L_1}{\log L_2 - \log L_1}\ \mathrm{g\ m^{-2} day^{-1}} \quad (5)$$

where W_2 and W_1 are the final and initial dry weights of aerial plant parts per unit area at the times t_1 and t_2, respectively, and L_2 and L_1 are the final and initial leaf area indices at respective times.

Photosynthetic rate (PR) was calculated by using LICOR machine (LI-6400XT) which is a portable photosynthesis system on physiologically active leaves of mustard.

Harvest index percentage was calculated by using the formula (6) given by Donald and Hamblin (1976):

$$\mathrm{HI}\ (\%) = \frac{Economic\,Yield}{Biological\,Yield} \times 100 \quad (6)$$

This study also pertained to assess the biochemical changes in leaves of mustard under different treatments of biofertilizer, chemical fertilizer and vermicompost and was evaluated in terms of total chlorophyll, sugar and proline content to enhance crop growth, metabolism and optimum protection against pathogens and pest. Physiologically active leaf (2nd and 3rd from the top) samples from ten different plants of each plot were plucked randomly for the determination of different biochemical parameters. Composite samples of leaves were crushed separately in a mortar with 10 ml of distilled water, poured in test tube and centrifuged at 10,000 rpm for 10 min. The supernatant was used for soluble sugar estimation by using anthrone reagent as described by Mc Cready et al. (1950). Chlorophyll content (mg g^{-1} fresh weight of leaves) was determined through organic solvent (80% acetone) extraction method as described by Arnon (1949). Proline content in leaves was determined by following the method of Bates et al. (1973).

2.9. Statistical analysis

Statistical analysis was done using the MINITAB software (version 8.0, Inc., Des Moines, IA, USA). Means of three replicates were taken. Means followed by the same letter within a treatment were not significantly different at the 5% level using Duncan's multiple range test (DMRT).

3. Results and discussion

In the present study, 25% reduction of NPK and combination with vermicompost (T4) proved significantly effective on various morpho-physiological and bio-chemical attributes studied at different growth stages.

3.1. Crop morpho-physiology

3.1.1. Leaf area index and photosynthetic rate

The data presented in Table 1 revealed greater LAI value shown by treatment T4 in all phases of both the experimental years. LAI values increased in the initial stage of development of crop i.e., from 30 to 45 DAS but gradually declined in the later stage of development from 45 to 60 DAS under all treatments. With maturity of crop growth, LAI increased due to newly emerged leaves and then it decreased gradually due to leaf senescence toward the maturity of the crop. A similar finding was reported by Banerjee et al. (2012a,b). Correspondingly, an enhancement in shoot and root fresh and dry weight was recorded at similar sampling stages (Mondal et al., 2014). Stimulating effects of biofertilizer application also increased greater amount of light interception by the crop plants which have contributed toward the vegetative growth of crop plants under all treatments (Aduloju et al., 2009). Greater light interception by the T4 treated plants leads toward higher rate of photosynthesis or photosynthetic rate (Table 6) which contributed significantly toward the vegetative growth of crop plants of B9 variety leading to higher LAI value. These findings are in line with some earlier findings in case of soybean crop (Aduloju et al., 2009) and sunflower plants (Gorttappeh et al., 2000).

3.1.2. Leaf area ratio

The effect of various treatment performances on leaf area ratio is shown in Table 2. LAR indicates how a system is efficient in growth and in broad sense it reflects the ratio of photosynthesizing to respiring material within the plant. The revealed

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Table 1 Leaf area index (LAI) values of mustard (*Brassica campestris* cv. B9) depending on vermicompost, biofertilizer and chemical fertilizer treatments during the winter cropping seasons of 2011–2012 and 2012–2013. Different letters within the same row indicate mean values statistically different at $p \leqslant 0.05$ as determined by LSD test. The data shown are means of three replicates.

	Leaf area index					
	2011–2012			2012–2013		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T1	0.37^{bc}	1.59^{d}	1.49^{ab}	0.13^{b}	6.68^{d}	5.54^{bc}
T2	0.81^{ab}	2.14^{bcd}	1.24^{ab}	0.15^{b}	8.30^{abc}	6.23^{bc}
T3	0.29^{c}	1.77^{cd}	1.01^{ab}	0.19^{ab}	7.94^{bcd}	5.82^{bc}
T4	1.08^{a}	4.32^{a}	1.65^{a}	0.22^{ab}	9.48^{a}	8.66^{a}
T5	0.71^{abc}	2.59^{bcd}	1.19^{ab}	0.21^{ab}	7.21^{cd}	4.68^{c}
T6	0.98^{a}	3.34^{ab}	0.88^{ab}	0.23^{ab}	8.84^{ab}	7.44^{ab}
T7	0.76^{abc}	2.95^{bc}	0.82^{b}	0.26^{a}	7.17^{cd}	6.57^{bc}
SEM ±	0.152	0.37	0.237	0.03	0.39	0.643
CD AT 5%	1.63	2.54	2.04	0.723	2.62	3.358
CV%	36.9	22.73	34.8	26.0	8.6	17.4
LSD AT 5%	0.467	1.14	0.732	0.092	1.21	1.981

Notes: Means followed by the same letter between treatments are not significantly different at 5% level using Duncan's multiple range test (DMRT). Means of three replicates are taken, where T1 – Full recommended dose of chemical fertilizer, T2 – 50% of recommended dose of chemical fertilizer + vermicompost (2.5 t ha^{-1}), T3 – 50% of recommended dose of chemical fertilizer + dried cow dung (2.5 t ha^{-1}), T4 – NPK (75% of full dose) + vermicompost (2.5 t ha^{-1}), T5 – NPK (75% of full dose) + dried cow dung (2.5 t ha^{-1}), T6 – NPK (75% of full dose) + vermicompost (2.5 t ha^{-1}) + *Azotobacter* and PSB (7.5 kg ha^{-1} each), T7 – NPK (75% of full dose) + dried cow dung (2.5 t ha^{-1}) + *Azotobacter* and PSB (7.5 kg ha^{-1} each). SEM, standard error of the means; CV, coefficient of variation; CD, critical difference; LSD, least significant difference; DAS, days after sowing.

Table 2 Leaf area ratio (LAR) values of mustard (*Brassica campestris* cv. B9) depending on vermicompost, biofertilizer and chemical fertilizer treatments during the winter cropping seasons of 2011–2012 and 2012–2013. Different letters within the same row indicate mean values statistically different at $p \leqslant 0.05$ as determined by LSD test. The data shown are means of three replicates.

	Leaf area ratio (m^2 g^{-1})					
	2011–2012			2012–2013		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T1	0.17^{b}	2.37^{c}	4.08^{d}	2.51^{a}	5.59^{cd}	2.63^{d}
T2	0.08^{bcd}	3.36^{c}	5.09^{cd}	2.05^{abc}	4.01^{e}	3.29^{d}
T3	0.06^{cd}	2.70^{c}	4.40^{d}	1.34^{c}	4.93^{de}	2.93^{d}
T4	0.28^{a}	7.03^{a}	7.68^{a}	2.01^{abc}	10.11^{a}	7.63^{a}
T5	0.01^{d}	3.37^{c}	5.58^{bcd}	1.47^{bc}	5.56^{cd}	4.59^{c}
T6	0.15^{bc}	6.37^{ab}	6.29^{abc}	2.21^{ab}	8.11^{b}	6.59^{b}
T7	0.08^{bcd}	5.37^{b}	6.94^{ab}	1.78^{abc}	6.49^{c}	5.57^{c}
SEM ±	0.03	0.36	0.47	0.24	0.45	0.33
CD AT 5%	0.76	2.53	2.88	2.04	2.8	2.41
CV%	17.5	14.5	14.3	21.6	12.1	12.1
LSD AT 5%	0.1	1.12	1.45	0.73	1.38	1.02

Note: For abbreviations, see footnote Table 1.

values indicated that in the later stage of development i.e., 45 and 60 DAS, T4 and T1 showed highest and lowest values respectively in both the experimental years. Maximum LAR values mean maximum biomass was deposited in the shoot and branches of the studied B9 mustard variety of this investigation.

3.1.3. Crop growth rate

CGR is a simple and important index of agricultural productivity on rate of dry matter production. Again treatment T4 showed the highest CGR values in two consecutive experimental years (Table 3). When LAI is large to intercept 95% of sunlight then plant gets optimum crop growth rate and also greater light interception stimulates CGR which in turn increases total dry matter and LAI. Greater LAI causes higher light interception which further enhances CGR and thus results in higher yield (Datta et al., 2012).

3.1.4. Leaf area duration

LAD can be defined as a measure of the ability of plant to produce and maintain leaf area and its whole opportunity for assimilation (Watson, 1947). Highest value of LAD in later

Table 3 Crop growth rate (CGR) values of mustard (*Brassica campestris* cv. B9) depending on vermicompost, biofertilizer and chemical fertilizer treatments during the winter cropping seasons of 2011–2012 and 2012–2013. Different letters within the same row indicate mean values statistically different at $p \leqslant 0.05$ as determined by LSD test. The data shown are means of three replicates.

	Crop growth rate (g m^{-2} day^{-1})			
	2011–2012		2012–2013	
	30–45 DAS	45–60 DAS	30–45 DAS	45–60 DAS
T1	2.38^{abc}	4.23^{c}	2.85^{c}	5.06^{e}
T2	3.50^{ab}	5.03^{bc}	3.58^{bc}	6.72^{d}
T3	3.42^{abc}	3.88^{c}	2.53^{c}	5.13^{e}
T4	3.74^{a}	10.54^{a}	5.01^{a}	10.49^{a}
T5	2.02^{bc}	6.75^{abc}	3.11^{bc}	8.11^{c}
T6	2.50^{abc}	9.09^{ab}	4.19^{ab}	9.21^{b}
T7	1.81^{c}	7.69^{abc}	2.94^{c}	7.99^{c}
SEM ±	0.49	1.24	0.34	0.29
CD AT 5%	2.94	4.66	2.45	2.24
CV%	15.39	16.51	17.2	6.6
LSD AT 5%	1.52	3.82	1.06	0.88

Note: For abbreviations, see footnote Table 1.

Table 4 Leaf area duration (LAD) values of mustard (*Brassica campestris* cv. B9) depending on vermicompost, biofertilizer and chemical fertilizer treatments during the winter cropping seasons of 2011–2012 and 2012–2013. Different letters within the same row indicate mean values statistically different at $p \leqslant 0.05$ as determined by LSD test. The data shown are means of three replicates.

	Leaf area duration			
	2011–2012		2012–2013	
	30–45 DAS	45–60 DAS	30–45 DAS	45–60 DAS
T1	4.74^{a}	6.79^{b}	4.71^{a}	7.59^{bc}
T2	3.42^{a}	7.97^{b}	4.89^{a}	6.59^{bc}
T3	5.4^{a}	7.12^{b}	5.98^{a}	6.24^{c}
T4	6.35^{a}	11.46^{a}	4.92^{a}	10.91^{a}
T5	3.91^{a}	8.33^{b}	5.64^{a}	5.99^{c}
T6	5.62^{a}	9.16^{ab}	5.10^{a}	8.39^{b}
T7	6.27^{a}	8.76^{ab}	5.52^{a}	6.95^{bc}
SEM ±	1.01	0.9	0.57	0.62
CD AT 5%	4.21	3.97	3.15	3.31
CV%	19.7	18.3	18.7	14.7
LSD AT 5%	3.11	2.77	1.74	1.97

Note: For abbreviations, see footnote Table 1.

stage of the crops maturity means the treatment performance of combined and balanced dose of fertilizers (Table 4). The enhanced rate of LAD on 30–45 DAS to 45–60 DAS may be attributed toward higher rate of dry matter accumulation by the crop plants under various treatments.

Table 5 Net assimilation rate (NAR) values of mustard (*Brassica campestris* cv. B9) depending on vermicompost, biofertilizer and chemical fertilizer treatments during the winter cropping seasons of 2011–2012 and 2012–2013. Different letters within the same row indicate mean values statistically different at $p \leqslant 0.05$ as determined by LSD test. The data shown are means of three replicates.

	Net assimilation rate (g m^{-2} day^{-1})			
	2011–2012		2012–2013	
	30–45 DAS	45–60 DAS	30–45 DAS	45–60 DAS
T1	0.41^{a}	0.32^{c}	0.1^{a}	0.17^{ab}
T2	0.55^{a}	0.89^{c}	0.12^{a}	0.13^{bc}
T3	0.45^{a}	0.67^{c}	0.11^{a}	0.09^{c}
T4	0.67^{a}	2.41^{a}	0.1^{a}	0.21^{a}
T5	0.42^{a}	0.97^{bc}	0.09^{a}	0.14^{bc}
T6	0.42^{a}	1.79^{a}	0.09^{a}	0.16^{ab}
T7	0.41^{a}	1.69^{ab}	0.08^{a}	0.15^{abc}
SEM ±	0.08	0.24	0.01	0.02
CD AT 5%	1.2	2.06	0.48	0.59
CV%	19.7	13.6	23.8	22.5
LSD AT 5%	0.25	0.75	0.04	0.06

Note: For abbreviations, see footnote Table 1.

Table 6 Photosynthetic rate of physiologically active leaves of mustard (*Brassica campestris* cv. B9) depending on vermicompost, biofertilizer and chemical fertilizer treatments during the winter cropping seasons of 2011–2012 and 2012–2013. Different letters within the same row indicate mean values statistically different at $p \leqslant 0.05$ as determined by LSD test. The data shown are means of three replicates.

	Photosynthetic rate (μmol m^{-2} s^{-1}) on 45 DAS	
	2011–2012	2012–2013
T1	19.15^{f}	22.58^{c}
T2	22.18^{d}	23.43^{abc}
T3	20.05^{e}	22.74^{bc}
T4	24.03^{a}	25.38^{a}
T5	23.12^{c}	24.12^{abc}
T6	23.65^{ab}	24.67^{ab}
T7	23.52^{bc}	24.50^{abc}
SEM ±	0.13	0.59
CD AT 5%	1.54	3.22
CV%	1.1	4.3
LSD AT 5%	0.42	1.82

3.1.5. Net assimilation rate

NAR is related to photosynthetic activities of leaf, i.e., rate of increase in dry weight of whole plant per unit leaf area. There is a considerable amount of variation in the NAR values of the seven applied treatments in the later stages of crop growth which can be due to the formation of siliquae that led to differential rate of supply of photo-assimilate toward the vegetative growth of crop plants (Table 5). During the siliquae formation stage the rate of photosynthesis in leaves decreases and siliquae increases (Miri, 2007). Steady increase in NAR value at the later stages of crop development can be explained on the basis of the fact that higher nutrient availability to crop plants in the presence of vermicompost, biofertilizer and chemical fertilizer caused more cell elongation in shoot and root development which lead to the progressive development of the CGR and NAR. Our findings corroborate the finding of Shukla and Warsi (2000).

3.1.6. Harvest index

In the current investigation there was considerable variation in the percent ratio of economic and biological yield of the crop i.e., HI value among the seven applied treatment combinations (Table 10). This is due to variable rate of translocation of assimilate toward grain development, leading to variability in yield and HI value among the seven applied treatments (Banerjee et al. (2012a,b)).

3.2. Bio-chemical

This study pertained to assess the biochemical changes in physiologically active leaves of mustard under different treatments of biofertilizer, chemical fertilizer and vermicompost and was evaluated in terms of chlorophyll and proline content to enhance crop growth, metabolism and optimum protection against pathogens and pest.

3.2.1. Total chlorophyll content

Regarding the effect of combined dose of chemical, vermicompost and bio-fertilizer, it was noted that all the combined doses

Table 7 Total chlorophyll contents of physiologically active leaves of mustard (*Brassica campestris* cv. B9) depending on vermicompost, biofertilizer and chemical fertilizer treatments during the winter cropping seasons of 2011–2012 and 2012–2013. Different letters within the same row indicate mean values statistically different at $p \leqslant 0.05$ as determined by LSD test. The data shown are means of three replicates.

	Total chlorophyll (mg g^{-1} fresh weight of leaves)					
	2011–2012			2012–2013		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T1	1.45^{b}	2.53^{b}	1.71^{ab}	1.61^{abc}	1.42^{b}	1.40^{ab}
T2	0.31^{c}	1.26^{c}	1.18^{b}	1.31^{c}	1.41^{b}	1.3^{ab}
T3	1.35^{b}	2.36^{b}	1.87^{a}	1.70^{ab}	1.4^{b}	1.43^{ab}
T4	2.21^{a}	3.26^{a}	2.03^{a}	1.93^{a}	1.92^{a}	1.56^{a}
T5	1.58^{b}	2.44^{b}	1.6^{ab}	1.37^{bc}	1.39^{b}	1.07^{ab}
T6	1.45^{b}	2.48^{b}	1.37^{ab}	1.41^{bc}	1.69^{ab}	1.27^{ab}
T7	1.23^{b}	1.96^{b}	1.38^{ab}	1.26^{c}	1.55^{b}	1.00^{b}
SEM ±	0.16	0.19	0.16	0.11	0.09	0.14
CD AT 5%	1.66	1.85	1.66	1.36	1.29	1.6
CV%	19.8	12.8	13.7	12.2	10.8	19.5
LSD AT 5%	0.48	0.53	0.39	0.33	0.29	0.45

Note: For abbreviations, see footnote Table 1.

Table 8 Total soluble sugar contents of physiologically active leaves of mustard (*Brassica campestris* cv. B9) depending on vermicompost, biofertilizer and chemical fertilizer treatments during the winter cropping seasons of 2011–2012 and 2012–2013. Different letters within the same row indicate mean values statistically different at $p \leqslant 0.05$ as determined by LSD test. The data shown are means of three replicates.

	Total soluble sugar (mg g^{-1} fresh weight of leaves)					
	2011–2012			2012–2013		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T1	5.85^{b}	6.52^{b}	11.00^{ab}	3.63^{c}	4.93^{c}	10.29^{bc}
T2	8.02^{ab}	9.21^{ab}	8.78^{b}	4.79^{bc}	7.26^{bc}	8.15^{c}
T3	6.52^{b}	7.97^{ab}	8.45^{b}	4.74^{bc}	6.79^{bc}	8.89^{c}
T4	12.34^{a}	13.71^{a}	15.95^{a}	8.51^{a}	16.32^{a}	17.20^{a}
T5	8.30^{ab}	9.48^{ab}	11.01^{ab}	3.71^{c}	8.96^{bc}	13.36^{abc}
T6	10.53^{ab}	12.74^{ab}	14.01^{ab}	7.22^{ab}	11.48^{ab}	15.84^{ab}
T7	9.65^{ab}	11.97^{ab}	12.08^{ab}	6.68^{ab}	8.30^{bc}	11.91^{abc}
SEM ±	1.55	1.87	1.91	0.77	1.65	1.89
CD AT 5%	5.21	5.72	5.79	3.68	5.39	5.75
CV%	14.22	13.37	11.9	15.67	14.06	11.23
LSD AT 5%	4.76	5.76	5.89	2.38	5.10	5.81

Note: For abbreviations, see footnote Table 1.

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of treatments increased the total chlorophyll content gradually over the control (T1). Application of vermicompost proved optimum (T4) at all stages of growth (Table 7). The productivity of any crops depends on the process of photosynthesis, which in turn depends on the chlorophyll content of leaves in plants (Banerjee et al. (2012a,b)). The significant variation in the level of total chlorophyll content in physiologically active leaves of mustard with respect to T1 can be due to variable rate of biosynthesis of chlorophyll and photosynthesis depending upon the genetic potential of the mustard against different treatment combinations and increased uptake of magnesium from the soil in the form of Mg^{+2} under the influence of vermicompost application. Beneficial effects of bacterial inoculation on increased chlorophyll content are due to higher availability of nitrogen to the growing tissue and organs supplied by nitrogen fixing *Azotobacter* species (Chandrasekhar et al., 2005), which also produced growth promoting substances resulting in more efficient absorption of nutrients through photosynthetic pigments and consequently the chlorophyll content increased (Hassan, 2009). The positive effect of N supply on the formation of chloroplasts during leaf growth also enhances chlorophyll content of leaves (Singh et al., 2014). In turn, the chloroplast formation leads to an increase in the lipid content of leaves and chloroplast constituents such as chlorophyll and carotene.

3.2.2. Total soluble sugar

Significant level of variation in total soluble sugar content in leaves among the different treatments was attributed toward the variable rate of photosynthesis leading to production of variable amount of photosynthate in the leaves along with adequate supply of nutrients (Table 8). Our findings were similar with the earlier findings on *Sideritis montana* (El-Sherbeny et al., 2005) and on *Iris* (Ali, 2005). Higher biosynthesis of chlorophyll and photosynthesis in flag leaf of plants under the vermicompost treated plots resulted toward higher level of total soluble sugar content of leaves. The results also reveal that biofertilizers have pronounced influence on biosynthesis of carbohydrates in leaves (Rao et al., 2007). These improved levels of sugar changes are of particular importance because of their direct relationship with such physiological processes such as photosynthesis, translocation and respiration. The sugar content in leaves increased up to 25% of reduced dose of nitrogenous chemical fertilizer and then decreased further with gradual increment of reduction of nitrogen fertilizer dose which was attributed toward higher rate of translocation of sugar transported from leaves to flowering parts and its subsequent utilization for the development of seeds under reduced supply of nitrogen to crop plants. This therefore indicates the role of nitrogen toward biosynthesis and accumulation of

Table 10 Harvest Index of mustard (*Brassica campestris* cv. B9) depending on vermicompost, biofertilizer and chemical fertilizer treatments during the winter cropping seasons of 2011–2012 and 2012–2013. Different letters within the same row indicate mean values statistically different at $p \leqslant 0.05$ as determined by LSD test. The data shown are means of three replicates.

	Harvest index	
	2011–2012	2012–2013
T1	54.82[ab]	63.35[c]
T2	59.42[ab]	57.92[c]
T3	58.07[ab]	86.99[b]
T4	71.27[a]	113.27[a]
T5	54.99[ab]	69.41[bc]
T6	64.98[ab]	86.31[b]
T7	54.25[b]	73.97[bc]
SEM ±	4.85	5.69
CD AT 5%	9.22	9.99
CV%	14.1	12.5
LSD AT 5%	14.93	17.54

Note: For abbreviations, see footnote Table 1.

Table 9 Proline contents of physiologically active leaves of mustard (*Brassica campestris* cv. B9) depending on vermicompost, biofertilizer and chemical fertilizer treatments during the winter cropping seasons of 2011–2012 and 2012–2013. Different letters within the same row indicate mean values statistically different at $p \leqslant 0.05$ as determined by LSD test. The data shown are means of three replicates.

	Proline (mg g^{-1} fresh weight of leaves)					
	2011–2012			2012–2013		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T1	7.24[ab]	6.36[b]	6.44[b]	4.39[a]	3.51[b]	3.83[b]
T2	4.85[b]	8.45[ab]	7.73[b]	2.15[a]	9.42[ab]	10.47[ab]
T3	4.03[b]	7.11[ab]	7.56[b]	3.38[a]	6.18[b]	3.29[b]
T4	13.18[a]	14.10[a]	14.7[a]	1.11[a]	14.81[a]	14.30[a]
T5	7.70[ab]	6.5[b]	6.71[b]	2.40[a]	7.46[b]	7.85[ab]
T6	10.54[ab]	11.43[ab]	11.13[ab]	1.70[a]	13.89[a]	12.64[ab]
T7	7.9[ab]	9.39[ab]	9.67[ab]	1.10[a]	8.93[ab]	10.2[ab]
SEM ±	2.14	2.19	1.59	1.09	1.9	2.80
CD AT 5%	6.12	6.2	5.27	4.38	5.77	7.01
CV%	18.46	16.35	13.79	15.08	15.02	17.86
LSD AT 5%	6.59	6.75	4.89	3.37	5.84	8.77

Note: For abbreviations, see footnote Table 1.

sugar in crop plants. Similar findings were reported by Setua et al. (2005) in mulberry leaves.

3.2.3. Proline content

The perusal of data revealed that proline content in leaves of the different studied treatments of mustard reflects the differential response of the seven treatments toward environmental stress and different levels of osmotic adjustment (Ozturk and Demir, 2002). 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 treatments of compost (Table 9). Our findings support the earlier findings of Sheteawi and Tawfik (2007).

4. Conclusions

Present study concludes that combined application of chemical fertilizer, biofertilizers and vermicompost reflects the variable morpho-physiological and biochemical response of mustard plants toward synthesis and accumulation of simple secondary metabolites and pigment content such as sugar, proline and chlorophyll respectively in leaves which therefore played a role in regulation of plant osmosis and crop plant metabolism leading to better crop growth and yield. Combined application of different fertilizers has enhanced LAI, CGR, NAR, PR, LAD and HI of mustard crop plants. Efficient utilization of applied inputs in a particular set of environments is reflected by crop growth rate and net assimilation rate which are in fact the gain in weight of community of plants per unit of land and time. Combined application of organic and inorganic fertilizers 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 led to the growth and yield attributes of crop plants. The present study will be useful to enhance crop production and it may combat global demand. We further infer from the study that at least 25% of NPK fertilizer amount can easily be substituted by vermicompost (T4) under this agroclimatic condition of old alluvial soil, Burdwan, India, which can help in increasing economic conditions of farmers and at the same time government has to spend less money to import chemical fertilizers from other countries.

Conflict of interest

There is no conflict of interest.

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