



Comparative Studies of Effects of Fertilizers and Manures on Biochemical Characteristics of Sesame (*Sesamum indicum* L.) Plant

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ABSTRACT

The present study was carried out to analyze biochemical parameters of *Sesamum indicum* L. by treating the plants with fertilizers and manures at University of Rajasthan, Jaipur. The experiment consisted of five treatments named as T₁- control (without any manure or fertilizer), T₂- urea as nitrogen fertilizer, T₃- urea + diammonium phosphate as N+P fertilizer, T₄- vermicompost, and T₅- a combination of vermicompost + biofertilizer. These were evaluated in a complete randomized design with four replications. Biochemical parameters taken for the study were- lipids, proteins, total soluble sugars, starch, phenols, and two antioxidant enzymes: peroxidase and catalase. Experimental results showed that chlorophyll-a, b, total chlorophyll, protein content, lipids, and peroxidase were observed maximum in vermicompost treated plants. Total phenols were observed increased in vermicompost + biofertilizer treatment. Therefore, they can be used to enhance the above parameters as an alternative of inorganic fertilizers under the arid conditions of Rajasthan. However, carotenoids, TSS, starch, and catalase were found higher in control plants. These parameters were not influenced significantly by fertilizer and manure treatments. It suggests that an optimized fertilizer rate is necessary to ensure agricultural sustainability in respect of synthesized food and antioxidants.

Key words: *Sesamum indicum* L., Fertilizers, Manures, Biochemical characteristics

Sesame (*Sesamum indicum* L.) is an important oilseed crop of the warm region of the tropics and sub-tropics and considered the most ancient oilseed crop grown in India (Wayase *et al.* 2014). It belongs to the family Pedaliaceae. Sesame has antioxidant value due to the presence of lignans (sesamin, sesamol) and tocopherol. Sesame seeds are rich in fatty acids like oleic, linoleic, palmitic, and stearic acids, also contains vitamins (vitamin E, vitamin A and B complex, niacin) and some other minerals (Choudhary *et al.* 2017).

For proper growth and optimal yield of any crop, nutrients must be required to plants in correct quantity and proportion. To fulfill these requirements, chemical fertilizers or organic manures are required (Sheikh and Ishaq 2016). Chemical fertilizers have been used to increase the

productivity of food crops by promoting plant growth but high inputs of agrochemicals can cause adverse environmental effects such as pollution, death of beneficial micro-organisms, residue accumulation in soil, and finally affect soil fertility. All these adverse effects of synthetic fertilizers lead to the search for more sustainable and eco-friendly agricultural methods. Organic manure provides soil organic matter and beneficial soil microbes, have importance as key factors in maintaining soil quality and crop production (Subash and Raftah 2016, Kurdiya *et al.* 2019). A new approach is biofertilizers are non-hazardous, environmentally friendly products. These have been used in sustainable agriculture to improve the plant nutrients present in soil (Youssef *et al.* 2010).

In Rajasthan, farmers face the low nutrient status of soil due to drought, lack of proper rain, excess use of fertilizers, and many other reasons. It affects the growth, quality and, productivity of crops and also soil properties. Considering all these factors, the present study was done to evaluate the

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effect of fertilizers and manures on biochemical quality parameters of sesame.

MATERIALS AND METHODS

The present study was conducted in the department of botany, University of Rajasthan, Jaipur. A complete randomized design (CRD) was adopted for the experiment. The seeds were sown in experimental pots (10 kg) in July 2017. The minimum and maximum temperatures during growing crops ranged from 20-30°C and 28-35°C respectively. The pots were irrigated thrice in a week and watering schedules same in all treatments. The experiments consist of five treatments named T₁, T₂, T₃, T₄ and T₅, each replicated four times. Treatments were: T₁- control (without any fertilizer or manure), T₂- 40 kg N ha⁻¹ (0.392 gm urea in 10 kg soil), T₃- 40 kg. N + 25 kg. P₂O₅ ha⁻¹, (0.392 gm urea + 0.247 gm DAP in 10 kg soil), T₄-Vermicompost 10t ha⁻¹ (45.45 gm in 10 kg soil), T₅- Biofertilizer + vermicompost. A double dose of recommended urea fertilizer was applied in two splits: one split at sowing time and another at flowering time. Ten-day old seedlings were used for estimation of two antioxidative enzymes viz. peroxidase, and catalase. After two months, fresh plant leaves were used for chlorophyll estimation. After completing the life-cycle of sesame plants, leaves were shade dried and powdered. This dry material was used for protein, phenol, carbohydrate, and starch estimation. Chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll were estimated by Arnon's method (1949). Protein content in leaves was measured by a method described by Lowry *et al.* (1951). Phenol content in leaf was measured by a method described by Bray and Thorpe (1954). Carbohydrate was estimated in leaf by phenol-sulphuric acid method (Dubois *et al.* 1951). Peroxidase and catalase were estimated by methods used by Putter and Aebi (1974) respectively.

The statistical analysis was done using R language version 3.5. One-way ANOVA was used to check the significant difference between the treatments at p<0.05 significant level. T-tests were employed to test the difference between the different variables in multiple treatments. SEM± and critical difference (CD) values were also calculated at 0.05 probability level.

RESULTS AND DISCUSSION

Biochemical parameters studied in the sesame plant were chlorophyll-a, chlorophyll-b, carotenoids, total chlorophyll, protein, total soluble sugars (TSS), starch, total phenols and two antioxidant enzymes: catalase and peroxidase. These parameters were studied in different fertilizer treated plants such as control (T₁), nitrogen fertilizer (T₂), N+P (T₃), vermicompost (T₄) and vermicompost + biofertilizer (T₅) (Table 1).

In sesame plants studied, the amount of chlorophyll-a was found higher in T₄ treatment (1.31±0.02 mg/gm) followed by T₂ (1.19±0.03 mg/gm) and least observed in T₃ treatment (1.04±0.03 mg/gm). Chlorophyll-b amount was observed maximum in T₄ treatment (0.46±0.01 mg/gm) followed by T₅ (0.40±0.03 mg/gm) as compared to other

treatments (Fig 1). Similarly, total chlorophyll was maximum in T₄ (1.79±0.02 mg/gm) and minimum in T₃ (1.41±0.02 mg/gm). From these experiments, chlorophyll a, chlorophyll b and total chlorophyll were observed maximum in vermicompost treated plants (1.31, 0.46 and 1.79 mg/gm fresh weight respectively) in comparison to other treatments. Similar observations were found by Manikandan and Thamizhiniyan (2016). They observed that chlorophyll content was observed maximum in compost treatment in the *Helianthus annuus* L. plant. The reason for increased chlorophyll content may be that availability of N, P, and K content was higher in compost treatment which might be due to rapid decomposition and mineralization in compost as compared to inorganic fertilizers.

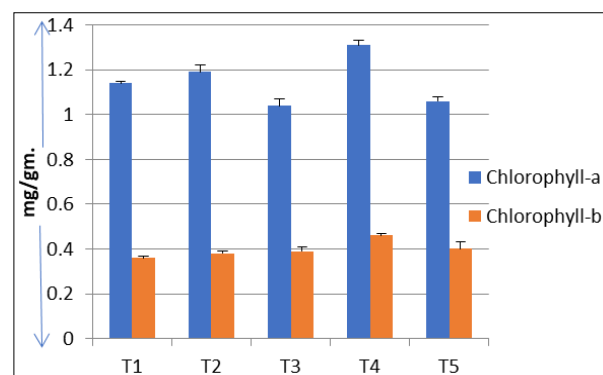


Fig 1 Histogram depicting chlorophyll-a and chlorophyll-b amount from different fertilizer treatments

The amount of carotenoid was observed higher in T₁ treatment (0.604±0.005 mg/gm) and lowest in T₃ treatment (0.418±0.001 mg/gm) (Fig 2). Carotenoids help plants to absorb light energy for use in photosynthesis. They also have an important antioxidant function of deactivating free radicals-single oxygen atom that can damage cells by reacting with other molecules (Sazalay 2015) Carotenoid was observed higher in T₁ treatment where no fertilizer or manure added. It may be due to nutrient deficiency stress.

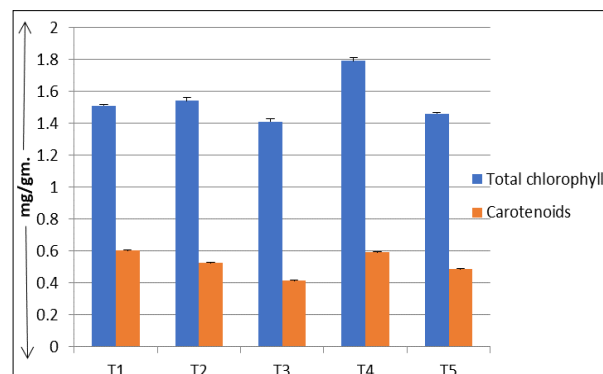


Fig 2 Histogram represents total chlorophyll and carotenoids amount from different fertilizer treatments

Protein content was analyzed 85.05±3.33, 98.06±1.71, 111.16±1.84, 129.94±14.80, 120.93±2.51 in T₁, T₂, T₃, T₄, and T₅ treatments respectively (Fig 3). These results show

that higher protein content was found in T₄ treatment and least was found in T₁ treatment. Protein content was observed higher in vermicompost treatment followed by the combined application of vermicompost + biofertilizer treatment. These results are in accord with Mathivanan *et al.* (2013) who concluded that vermicompost consist of macronutrients and micronutrients and the uptake of these nutrients have a positive effect on plant nutrition, photosynthesis, and chlorophyll content of the leaves. An increase in protein content was also reported by Gowda *et al.* (2008) in the wheat crop grown under vermicompost application.

Total soluble sugars (TSS) were found maximum in T₁ (12.86±0.90 mg/gm) followed by T₄ treatment (12.66±0.41 mg/gm) and minimum was observed in T₂ treatment (10.33±1.40 mg/gm) (Fig 4). Elhanafi *et al.* (2019) found in their study that increased nitrogen fertilizer had an adverse effect on the oil and sugars in sesame seeds. TSS content was negatively influenced by nitrogen supply. In our study, in T₂ treatment, double dose RDF of nitrogen fertilizer was added; it may have an adverse effect on TSS content in sesame leaves and observed the least value as compared to others. But in T₄ treatment, vermicompost releases nitrogen slowly in soil, so it may be possible that nitrogen not available in readily form, may not have an adverse effect on

TSS and its value was found higher in T₄ than other inorganic treatments, similarly, in T₁ treatment (control) where no fertilizer or manure added, has maximum TSS value followed by T₄ treatment. Other findings suggest that nitrogen is a direct factor that regulates carbon balance that is the basic element for sugar conservation. Sugar content was found to decrease with increasing nitrogen levels. Sugar is involved in many chemical reactions to produce energy (Xia and Cheng 2004).

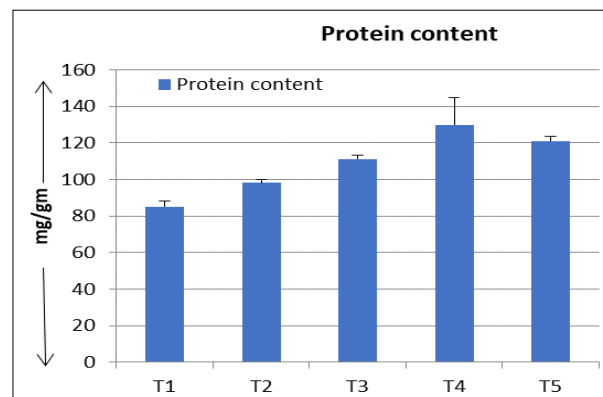


Fig 3 Histogram depicting protein amount from different fertilizer treatments

Table 1 Showing comparative studies on biochemical characteristics of sesame plant

Treatment	Chlorophyll-a (mg/gm)	Chlorophyll-b (mg/ gm)	Total chlorophyll (mg/ gm)	Carotenoids (mg/gm)	Protein (mg/gm)
T ₁	1.14± 0.01	0.36± 0.01	1.51±0.01	0.604±0.005	85.05± 3.33
T ₂	1.19± 0.03*	0.37± 0.01 ^{NS}	1.54±0.02 ^{NS}	0.525±0.004***	98.06± 1.71**
T ₃	1.04± 0.03 ^{NS}	0.39± 0.02 ^{NS}	1.41±0.02**	0.418±0.001**	111.16±1.84***
T ₄	1.31± 0.02***	0.46± 0.01**	1.79±0.02***	0.591±0.03*	129.94±14.80***
T ₅	1.06± 0.02*	0.40± 0.03*	1.46±0.01**	0.489±0.003***	120.93±2.51***
SEm±	0.04	0.01	0.01	0.002	4.02
CD (0.05)	0.13	0.03	0.04	0.006	12.84

.....Table 1 Continued.....

Treatment	TSS (mg/gm)	Starch (mg/gm)	Total phenols (mg/gm)	Lipids (mg/gm)	Catalase (µml/l/g/fw)	Peroxidase (µml/l/g/fw)
T ₁	12.86± 0.90	6.96±0.68	1.45±0.01	35± 6.24	1.54±0.48	1.82±0.66
T ₂	10.33± 1.40 ^{NS}	5.3±0.1*	1.07± 0.02***	37.6±7.31 ^{NS}	1.0±0.22 ^{NS}	1.52±0.59 ^{NS}
T ₃	12.01± 1.58 ^{NS}	5.63± 0.15*	1.22±0.02***	28.3±5.66 ^{NS}	0.82±0.12 ^{NS}	1.90±0.43 ^{NS}
T ₄	12.66± 0.41 ^{NS}	5.56± 1.80 ^{NS}	1.21± 0.02***	46.0±6 ^{NS}	0.78±0.08 ^{NS}	2.24±0.44 ^{NS}
T ₅	10.93±2.38 ^{NS}	4.6±0.7*	1.5±0.01*	42.0±11.13 ^{NS}	0.93±0.24 ^{NS}	1.60±0.77 ^{NS}
SEm±	0.86	0.53	0.01	4.35	0.22	0.39
CD (0.05)	NS	NS	0.039	NS	NS	NS

***=p 0.001, **=p 0.01, *=p 0.05, NS=Non significant

Starch content was estimated 6.96±0.68, 5.3±0.1, 5.63±0.15, 5.56±1.80, and 4.6±0.7 mg/gm. dry weight in T₁, T₂, T₃, T₄, and T₅ treatments respectively (Fig 4). A higher amount of starch was found in T₁ treatment and least in T₅ treatment. Experiments showed that total soluble sugars and starch were observed maximum in T₁ treatment as compared to treated plants. Similar results were also found by Hirano *et al.* (2005), Wang *et al.* (2006) who worked on rice plants and Chen and Cheng (2003) in grapes (*Vitis labrusca* L.).

They observed from their studies that starch and soluble sugars can accumulate with low nitrogen supply. Braun *et al.* (2017) analyzed starch accumulation in potato leaves, at conditions of low nitrogen. The relationship starch/NRS is indicative of carbon partitioning in plants, which has increased as doses of nitrogen were reduced. They stated from their experiments that at poor conditions of nitrogen (N) in the soil, potato plants may accumulate starch in leaves and be indicative of nitrogen nutritional stress. Cruz

et al. (2003) reported that this behaviour is due to a deficiency in the capacity of exporting sugar simultaneously to the stimulus for the formation of starch in cassava. They found that total soluble sugars and non-reducing sugars had their contents increased and reducing sugars as well as starch decreased as nitrate supply increased in the solution. The reason may be behind that the accumulation of soluble sugar under low nitrogen fertilizer might be due to a reduction in the sink size of the plant when nitrogen is limited; hence reducing the translocation of carbohydrate to the other plant parts (Meyer *et al.* 2006).

The amount of total phenols was observed maximum in T5 treatment (1.50 ± 0.015 mg/gm) and observed minimum in T2 treatment (1.07 ± 0.02 mg/gm) (Fig 4). Phenolic compounds are secondary plant metabolites and naturally present in almost all plant materials. These compounds can delay or inhibit the oxidative damage caused by free radicals such as superoxide anion radicals and non free-radical species such as H_2O_2 and singlet oxygen, by inhibiting the initiation or propagation of oxidative chain reactions (Alizadeh *et al.* 2010). Asami *et al.* (2003) reported that organic fertilizer alone did not stimulate the biosynthesis of phytochemicals as phenolics in strawberries and lettuce. These results are in accord with our results. Our results also

agree with Banerjee *et al.* (2012), who found in their study the maximum accumulation of phenol in leaves in biofertilizer and cycocel treated plots. Higher phenol content in leaves of biofertilizer treated plots might be attributed to the use of accumulated nitrate in plants thus enabling it to use more carbohydrates for structural growth under the influence of biofertilizer application (Hanafy *et al.* 2000). In other studies, it was considered that the concentration of phenol and proteins are negatively co-related (Jones and Hartley 1999). The reason may be behind that phenylalanine which is a key substance in the synthesis of phenols, is preferentially applied to protein chain synthesis rather than phenols (Li *et al.* 2008).

Lipid content was found maximum in T4 treatment (46.0 ± 6) followed by T5 treatment (42.0 ± 11.13) and observed minimum in T3 treatment (28.3 ± 5.66) (Fig 5). Lipid content was not significantly affected ($p > 0.05$). The positive effect of nitrogen supply on the formation of chloroplasts during leaf growth also increases the chlorophyll content of leaves (Singh *et al.* 2016). In turn, the chloroplast formation leads to an increase in the lipid content of leaves and chloroplast constituents such as chlorophyll and carotene (Mondal *et al.* 2017).

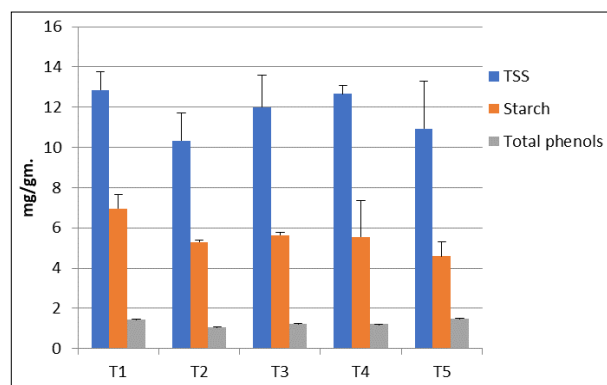


Fig 4 Histogram representing TSS, Starch and total phenols from different fertilizer treatments

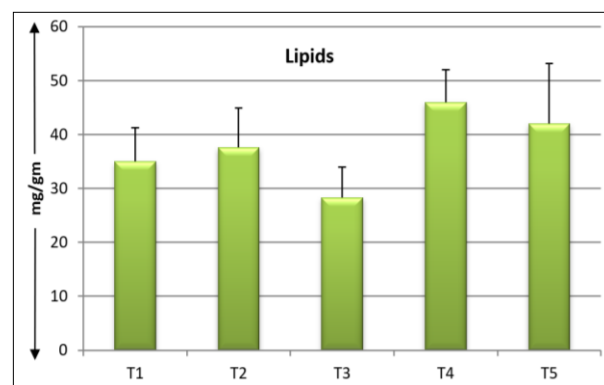


Fig 5 Histogram showing lipids amount from different fertilizer treatments

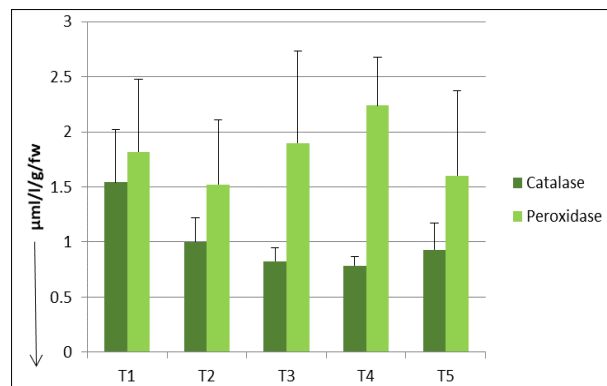


Fig 6 Histogram showing catalase and peroxidase concentration from different fertilizer treatments

Antioxidant enzymes (catalase and peroxidase) were estimated 1.54 ± 0.48 and 1.82 ± 0.66 in T1 treatment, 1 ± 0.22

and 1.52 ± 0.59 in T2 treatment, 0.826 ± 0.12 and 1.9 ± 0.43 in T3 treatment, 0.786 ± 0.08 and 2.24 ± 0.44 in T4 treatment, and 0.93 ± 0.24 and 1.60 ± 0.77 $\mu\text{m/l/g/fw}$ in T5 treatment. The amount of catalase was more in T1 treatment and a decreased amount was observed in T4 treatment. Peroxidase enzyme was found maximum in T4 treatment and minimum observed in T2 treatment. Catalase and peroxidase both were not significantly ($p > 0.05$) affected. Ahmed *et al.* (2010) reported that the application of organic manures and biofertilizers increase catalase and peroxidase activities in sorghum. They concluded in their study that this might be due to mineral fertilization can supply control plants greater amounts of nitrogen in a brief period to improve metabolism with no need of additional antioxidant enzymes, whereas in case of plants that receive the organic fertilizers face a condition of slow release of available nitrogen. That might be considered as adverse environmental conditions due to nutrient deficiency where the role of the antioxidant

enzymes support plants to become more tolerant against the proposed disturbances in different plant physiological process (Siavoshi and Laware 2013). In our study, catalase activity was more in T₁ (control) treatment, maybe nutrient deficiency stress, due to which plant accumulates high catalase when plant faces nutrient deficiency as seen in control.

Antioxidant levels in *Brassica rapa* seemed to decrease as the fertilizer rate increased, especially under conventional fertilization (Zhao *et al.* 2006, Sheikh and Ishak 2016). It was observed that the use of chicken dung (organic fertilizer) resulted in higher production of secondary metabolites and increased antioxidant activity compared to the use of NPK (inorganic fertilizer) (Ibrahim *et al.* 2013).

From our study, it was concluded that vermicompost

help to enhance chlorophyll content, proteins, lipids, and peroxidase in sesame plant under arid conditions of Rajasthan. The combined application of vermicompost and biofertilizer is beneficial for total phenols elevation, that has a major role in the prevention of oxidative damage. Whereas carotenoids, TSS, starch, and catalase were not influenced by fertilizer treatments may be due to nutritional stress. It suggests that an optimized fertilizer rate is necessary to ensure agricultural sustainability with respect to synthesized food and antioxidants.

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