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EFFECT OF VERMICOMPOST ON BIOCHEMICAL CONTENT OF TWO INDIAN GREEN LEAFY VEGETABLES

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ABSTRACT: Nowadays, the organic way of farming gains much more important and the people believe that the production of plants with organic farming has more nutrients and a lot of health benefits. In this, the green leafy vegetables occupy an important place among the food crops as they provide an adequate number of vitamins and minerals for humans. *Solanum nigrum* L. and *Trigonella foenum-graecum* L. are commonly used leafy vegetables in South India and also have so much of medicinal uses. The present investigation aims to identify the effect of different concentration of vermicomposting and biofertilizers on the biochemical content of both leafy vegetables. A total of five experimental models were designed T₀ (control) T₁ (vermicompost -15gm), T₂ (vermicompost -20gm) T₃ (vermicompost -15gm + Azospirillum) and T₄ (vermicompost -15gm + Azotobacter). Among all treatments, the vermicompost -15gm + asospirillum supplemented seedlings were absorbed significantly more protein, carbohydrates and amino acid content, whereas the chlorophyll content was recorded more in 20g of vermicompost treated seedlings of both crops.

INTRODUCTION: India is an agricultural country, majority of the Indians are engaged directly or indirectly on agriculture and agriculture related activities. Agriculture can be defined as the practice of crop and animal production on planned land units and peoples cannot survive in large numbers without fruitful agriculture¹. In today's era, heavy dosage of chemical fertilizers and pesticides are being used by the farmers to get better yield of various field crops, which decreases the soil fertility and causes so many health problems².

Therefore, farmers are in need of good, viable and ecofriendly alternative system of farming, which is called organic farming used with vermicompost and organic bio fertilizers to ensure the soil fertility and high nutrient crops without cause any side effect to human being^{3,4}.

The tenet "Let food be thy medicine and medicine be thy food" advocated by Hippocrates nearly 2500 years ago is getting renewed awareness. In traditional societies, nutrition and health care are strongly interconnected and many plants have been consumed both as food and for medicinal purposes^{5, 6}. Green leafy vegetables occupy an important place among the food crops as these provide adequate amount of many vitamins and minerals for humans. They are rich source of carotene, ascorbic acid, riboflavin, folic acid and minerals like calcium, iron, phosphorus and other phytochemicals⁷.

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Solanum nigrum L. is a common medicinal plant and a leafy vegetable belonging to the family Solanaceae. This is generally called as black nightshade. The leaf and fruits are used as traditional medicines with high neutraceutical, antiseptic, antidysentric and antidiuretic properties and it also used for the treatment of many skin diseases^{8, 9}. The *Trigonella foenum-graecum* is usually known as fenugreek belonging to the family Fabaceae. The leaves and seeds of fenugreek have used in food items as a flavoring agent since ancient times. Fenugreek is one of the oldest medicinal herbs used to stimulate digestive and metabolic process¹⁰.

MATERIALS AND METHODS:

Plant Material Collection: Pots of 4 kg capacity (25 cm × 22 cm) were individually filled with growth medium containing soil + sand (1:1 ratio) along with supplemented substrate for different treatments. The treatment details are as below:

- To- Control
- T1- vermicompost (15 gm)
- T2- vermicompost (20 gm)
- T3.-vermicompost (15 gm) + Azospirillum
- T4- vermicompost (15 gm) + Azotobacter

Seed Sowing and Maintenance of Experimental Set up:

Fifteen seeds of each test plants (*S. nigrum* and *T. foenum-graecum*) were sown with equal spacing between the seeds at uniform depth of 3 cm in each treatment pots individually after moistening the soil and ten replications were maintained. After the germination, the plants were collected for identification (*S. nigrum* (AU-207/2016-17) and *T. foenum-graecum* (AU-211/2016-17) and herbarium specimen were prepared and deposited for future reference in Department of Botany, Avinashilingam University, Coimbatore. Healthy leaves of both plants were collected on 30, 45 and 60 days interval and subjected to following biochemical parameters.

Biochemical Analysis: The photosynthetic pigments like chlorophyll a, chlorophyll b, and total chlorophyll and biochemical contents such as proteins, carbohydrates and amino acids were analyzed.

Estimation of Protein: Five hundred mg of plant material (leaf) were weighed and macerated in the pestle and mortar with 10 ml of 20% trichloroacetic

acid. The homogenate was centrifuged for 15 min at 600 g the supernatant was discarded. The pellet, 5 ml of 0.1N NaOH was added and centrifuged for 5 min. The supernatant was saved and made up to 10 ml of 0.1N NaOH. This extract was used for protein estimation. One ml of extract was taken in a 10 ml test tube and 5 ml of reagent C was added. The solution was mixed and kept in darkness for 10 min. Later 0.5 ml of Folin- phenol reagent was added and the mixture was kept in dark for 30 min. The sample was read at 660 nm in a UV spectrophotometer.

Estimation of Carbohydrate: 100 mg of sample were hydrolyzed in a boiling test tube with 5 ml of 2.5 N HCl in a boiling water bath for a period of 3h. It was cooled at room temperature and solid sodium carbonate was added until effervescence created. The content was centrifuged and the supernatant was made to 100 ml by using distilled water. From this 0.2 ml sample was pipetted out and made up the volume to one ml of distilled water. Then 4 ml of anthrone reagent was added and heated for eight minutes in a boiling water bath. Then it was cooled rapidly and the green colour developed was read at 630 nm.

Estimation of Free Amino Acid: Five hundred mg of leaf was macerated in the pestle and mortar with 10 ml of 80% ethanol. The homogenate was centrifuged at 800 g for 10 min and the supernatant was taken. The extraction was repeated four to five times and the supernatants were combined. An appropriate volume (5 - 10 ml) of this ethonolic extract was evaporated to dryness on a boiling water bath and the residue was dissolved in 5 ml of 0.2 M citrate buffer (pH 5.0). The above sample (2 ml) was taken in a test tube and 1 ml of ninhydrin reagent (4% ninhydrin in methyl cello solve and 0.2 M acetate buffer in the ratio of 1:1) was added to it. The samples were boiled for 20 min and cooled; the volume was made up to 10 ml with distilled water. The absorbance was read at 570 nm in a UV- spectrophotometer. Total free amino acids were calculated from a standard curve prepared against glycine (0 - 100I_g).

Estimation of Chlorophyll Content: Three grams of fresh leaves were blended and then extracted with 80% acetone and left for 15 min for extraction. The liquid portion was decanted into

another test tube and centrifuged at 2,500 rpm for 3 min. The supernatant was collected and the absorbance was taken at 645 nm and 663 nm using a spectrophotometer.

The amount of chlorophyll present in the extract mg chlorophyll/gram tissue were calculated by using the following equations.

$$(i) \text{ mg chlorophyll 'a' / g tissues} = 12.7 A_{663} - 2.69 A_{645} \times V / 1000 \times W$$

$$(ii) \text{ mg chlorophyll 'b' / g tissues} = 22.9 A_{645} - 4.68 A_{663} \times V / 1000 \times W$$

$$(iii) \text{ mg total chlorophyll / g tissues} = 20.2 A_{645} + 8.02 A_{663} \times V / 1000 \times W$$

Where, A = Absorbance at specific wave lengths, V= Final volume of chlorophyll extract in 80 per cent acetone, W = Fresh weights of tissue extracted

RESULTS AND DISCUSSION: Protein is a reserve food material, which is utilized for the growth and development of seedlings¹¹. The result on the effect of various dose of vermicompost on biochemical parameters of *S. nigrum* and *T. foenum-graecum* was presented in **Fig. 1** and **2**. The highest protein, carbohydrates and amino acids were recorded in vermicompost -15gm + Azospirillum supplemented seedlings when compare to control (**Fig. 1A, B & C** and **2A, B and C**). Increase in nitrogen content normally associated

with increase in protein content and believed to stimulate plant nutrient uptake and metabolism have influenced the protein synthesis¹². Similar results were observed in Shozeb and Aruna¹³ in vermicompost + biofertilizer treated seedling of *Glycine max* and *Vigna mungo*. The application of recommended doses of organic manures like vermicompost has significantly increased the protein content of mulberry leaves¹⁴.

In the present study, the application of different concentration and combination of vermicompost on chlorophyll a, chlorophyll b and total chlorophyll content of *S. nigrum* and *T. foenum-graecum* were estimated **Fig. 1D** and **2D**. The highest chlorophyll content was recorded in 20 g of vermicompost treated seedlings of both test crops. Subler *et al.*,¹⁵ observed that increase in chlorophyll content in response to vermicompost was observed at early stages of marigold. The increased chlorophyll content due to the application of vermicompost in lettuce was observed by Ali *et al.*¹⁶ The increasing chlorophyll content was due to the presence of microorganisms in the vermicompost, which colonize in the rhizosphere and stimulate the plant growth and biochemical content. Biochemical parameters of protein, carbohydrates, amino acid and chlorophyll contents found higher in bio fertilizer enriched vermicompost treatment. This is due to nutrient content of organic manure.

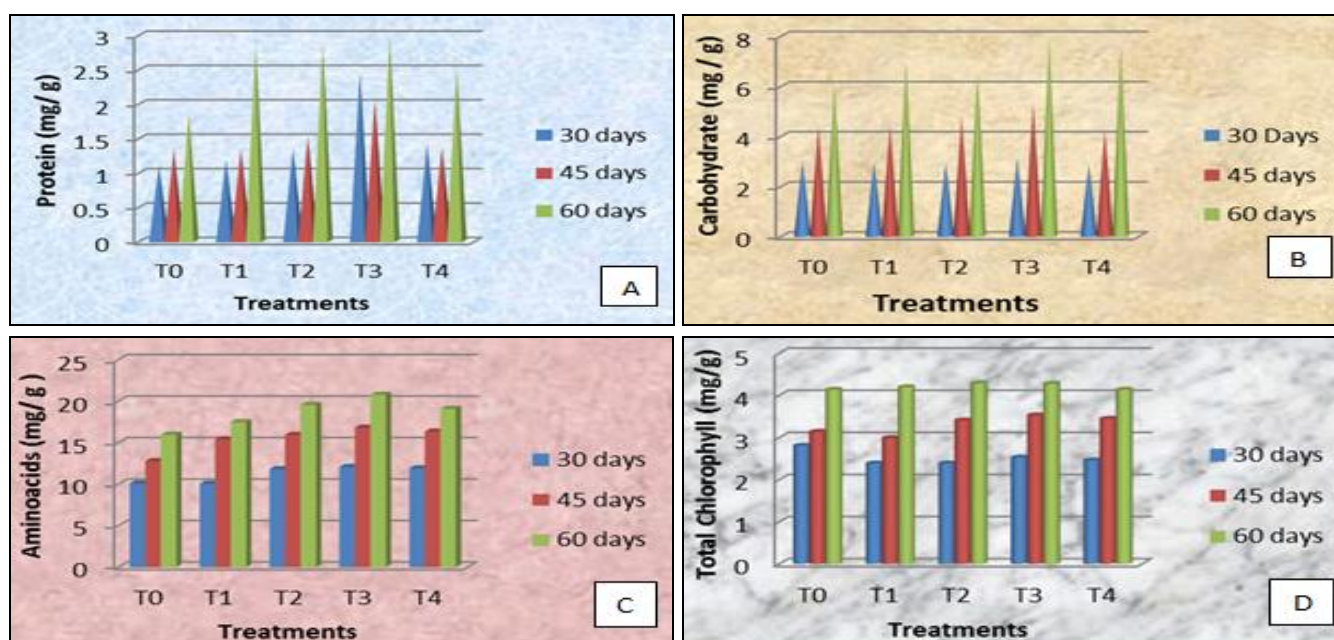


FIG. 1: EFFECT OF VERMICOMPOST ON (A) PROTEIN (B) CARBOHYDRATE (C) AMINOACIDS AND (D) TOTAL CHLOROPHYLL CONTENT OF SOLANUM NIGRUM L.

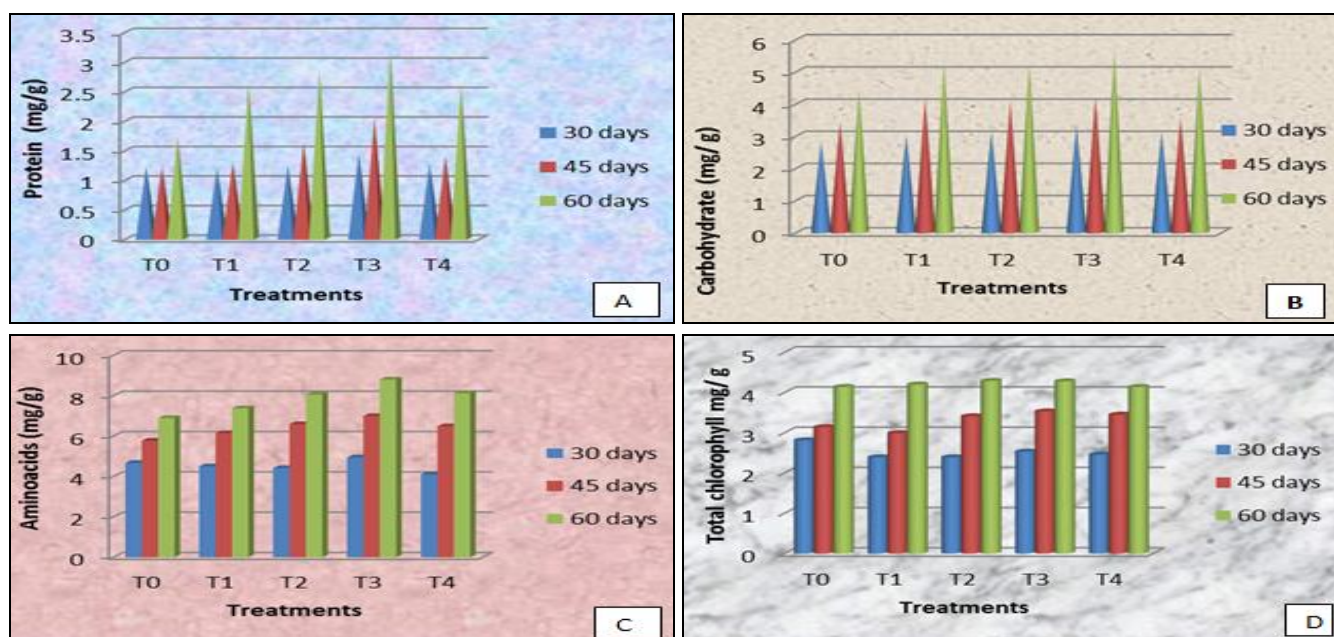


FIG. 2: EFFECT OF VERMICOMPOST ON (A) PROTEIN (B) CARBOHYDRATE (C) AMINOACIDS AND (D) TOTAL CHLOROPHYLL CONTENT OF *TRIGONELLA FOENUM- GRAECUM* L.

CONCLUSION: Vermicompost is always having a good source of plant growth promoting substances. A close perusal of the data obtained for the above-mentioned results reveals that the vermicomposting is one of the novel techniques used to get rid of the menace caused by organic wastes and vermicompost along with bio fertilizers, have tremendous scope to wrest the present day agriculture out of food and nutrition crisis. Hence, the present investigation confirmed that the usage of vermicompost alone and bio fertilizer with vermicompost significantly increases the biochemical content of *Solanum nigrum* and *Trigonella foenum-graecum*.

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CONFLICT OF INTEREST: The authors have declared no conflicts of interest

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