EFFECT OF ORGANIC AND INORGANIC FERTILIZER ON GROWTH, PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF SOLANUM NIGRUM L.

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ABSTRACT

The application of organic and inorganic fertilizer to the soil is considered as good agricultural practice because it improves the fertility of the soil and plant quality. The objective of this study is to compare the effect of organic (vegetable waste, cattle dung) and inorganic fertilizer (NPK) on Solanum nigrum. The experiment was arranged in a randomized design in three replicates. Parameters assessed include leaf area, height, leaf length, nutrient composition, phytochemicals and antioxidant activity on leaf of Solanum nigrum. The application of cattle dung and vegetable waste gave plants with the greatest plant height, leaf area and surface length. The mean values recorded for these parameters were 38±3.16, 10.5±2.18cm & 5.5±1.58cm for organic fertilizer and 36±3.14 cm, 8.0±1.12cm & 4.0±1.48cm for inorganic fertilizer. Organic manure produced higher effect on carbohydrate, protein, amino acid, vitamin c and chlorophyll content in the leaf of Solanum nigrum when compared with NPK fertilizer. Mean values recorded for the above parameters in leaf sample were 30.0±2.41, 1.93±1.93, 3.31±0.65, 2.53±0.72, 1.24±0.53mg/100g compared with values of 10.0±2.26, 1.25±1.62, 2.0±0.52, 2.26±0.80, 1.98±0.40mg/100g respectively for inorganic fertilizer. Effect of organic fertilizer in plant phytochemicals was higher than that of inorganic fertilizer. Values obtained for alkaloids, flavonoids, tannins, saponins and total phenol in organic treated leaf samples were 0.21±0.80, 0.9±0.53, 0.52±0.16, 0.34±0.36, 0.26±0.18mg/100g, respectively as against values of 0.2±0.32, 0.5±0.46, 0.4±0.12, 0.29±0.26, 0.8±0.14mg/100g for inorganic fertilizer. Organic fertilizer treated plants have higher antioxidant activity than the inorganic fertilizer. Mean values recorded for leaf sample...
in organic fertilizer were 64.21±15.81, 35.42±12.80mg/100g respectively corresponding mean values for inorganic leaf sample were 42.12±14.72, 25.42±7.90mg/100g. The experimental results of this study have showed that organic fertilizer produced higher effect on Solanum nigrum leaf when compared with inorganic fertilizer. High amounts of phytochemicals, nutrients and antioxidants recorded in this study gives preference to the use of organic than inorganic fertilizer.

**KEYWORDS:** Solanum nigrum, Nutritional status, phytochemicals & *In vitro* antioxidant activity. Organic and inorganic fertilizer.

**INTRODUCTION**
Declining soil fertility resulting from continuous cultivation of small holder farms and escalating cost of imported fertilizers and the need to conserve and build natural resource capital and biodiversity, has led to renewed interest in the use of local nutrient resources for soil fertilizer management.\(^1\) The use of organic matter such as animal manures, human waste, food wastes, yard wastes, sewage sludges and composts has long been recognized in agriculture as beneficial for plant growth and yield and the maintenance of soil fertility. The new approaches to the use of organic amendments in farming have proven to be effective means of improving soil structure, enhancing soil fertility and increasing crop yields. Organic matter are excellent source of plant-available nutrients and their addition to soil could maintain high microbial populations. Crop yields have increased with the corresponding improvements in soil quality from additions of organic matter. Significant yield increases using mulches from coffee husks and increases in productivity using animal manures and hay residues have been reported. Their important roles in the soil and their potentially positive effect on crop yields have made organic amendments a valuable component of farm fertilization and management programs in alternative agriculture.\(^2\)

The increasing global interest and expanding market of herbal drugs have led to their introduction into cultivation to meet the demand at reasonable economic price. Cultivation of medicinal plants can also facilitate in maintaining standard-in quality, potency and chemical composition of the produce. While are in constant endeavour to increase production, seldom the issue of quality of crops is studied. In medicinal plants, the quality of end product is very important; the secondary metabolites from medicinal and aromatic plants are valued in commerce. Biofertiliser, domestic and industrial waste application are commonly employed upgradation technique to medicinal and aromatic plants.\(^3\)
Solanum nigrum is an important leafy vegetable. It has been traditionally used as analgesic, antispasmodic, antiseptic, antidysentric, emollient, diuretic, soporific, laxative, anticancer, anti-ulcer and disorders of neuro-vegetative system. This medicinal value is attributed to the alkaloidal contents of the plant. In India Solanum nigrum mixed with other herbal medicine has shown to be hepatoprotective in cirrhotic patients. This protective effect can be attributed to the diuretic, antiflammatory, anti-oxidative and immune modulating properties of the component herbs. The fruits extracts of Solanum nigrum have anti-tumor and neuropharmacological properties and can be used as an anti-oxidant and cancer chemoprevention matter. Hence, the present study was taken to investigate the effect of organic and inorganic fertiliser on growth, secondary metabolites and antioxidant status of Solanum nigrum L.

MATERIAL AND METHODS
The experiment was carried out at the D.G.G.Arts College (W) in Mayiladuthurai. The study was carried out between November 2014 to February 2015 in the green house in experimental pots. The experiment was conducted using 5-litre pots containing 5kg of soil. Cattle dung and vegetable waste compost were applied at the rates of 1.5kg per 5kg of soil one week before sowing, while NPK fertilizer was applied at the rate of 3g per 5kg of soil three weeks after sowing. Ten viable seeds of Solanum were planted in each of the pots. Harvesting was done until the 8th week of plant age. The following parameters were observed in leaf of organic and inorganic treated Solanum nigrum plant.

Nutritional Analysis
Estimation of carbohydrate
1g of dried and powdered sample was extracted with 10ml of distilled water in water bath for 15min. The supernatant was used as the test sample. 1ml of the supernatant was taken and 4ml of anthrone reagent was added to it then boiled for 10min and then cooled at room temperature. Absorbance was measured at 620nm wavelengths. Carbohydrate was reported as mg/g of dry weight.

Estimation of protein
1.0gram of leaf powder was ground using pestle and mortar with 1.0 ml of phosphate buffer. These samples were kept overnight for complete extraction of protein. These were centrifuged for 20 minutes. The supernatant is used for protein analysis and the pellet is discarded. To 1.0 ml of supernatant from above is added 5.0 ml of alkaline copper sulfate
reagent and thoroughly mixed. Allowed to stand for ten minutes and then add 0.5 ml of Folin’s reagent. In order to develop colour this is kept standing for 30 minutes. This was followed by recording absorbance in colorimeter at 660 nm, against a blank. The blank is prepared by taking 1.0mL of 0.5 M NaOH in place of sample in cuvette. Bovine serum albumin is used to draw a standard curve and the amount of proteins in samples are estimated.

**Estimation of amino acids**

500mg of plant materials were weighed and macerated with a pestle and mortar with 10ml of 80% ethanol. The homogenate was centrifuged for 10minutes at 800g. The supernatant was saved and the extract was used for the estimation of amino acids. 1ml of the extract was pipetted out into a test tube. A drop of methyl red indicator was added. The sample was neutralized with 1ml of 0.1N sodium hydroxide. To this, 1ml of ninhydrin reagent was added and mixed thoroughly. The content of the test tube was heated for 20minutes in a boiling water bath. 5ml of the diluents solution was added and heated in water bath for 10minutes. The test tubes were cooled and the contents were mixed thoroughly. Blank was prepared with 1ml of distilled water (or) ethanol. The absorbance was read at 570nm in a colorimeter.[10]

**Determination of ascorbic acid**

The ascorbic acid content was measured using a modified method of.[11] The fresh leaf samples (1g) were extracted in 1% of phosphate-citrate buffer, pH 3.5 using a pestle and mortar. The homogenate was filtered. The filtrate was added to 1.7mM 2,6 dichloroindophenol (2,6-DCPIP, 1mL) in a 3ml cuvette. The absorbance at 520nm was read within 10min of mixing the reagents. The extraction buffer was used as a blank. L Ascorbic acid was used as a standard. Ascorbic acid was recorded as mg/g L-ascorbic acid in fresh leaves.

**Estimation of chlorophyll**

0.5gm fresh plant material was homogenized in 20ml of 80% chilled acetone with the help of mortar and pestle in dark. A pinch of MgCO₃ powder added. The extract filtered through Whatman no.1 filter paper. Final volume of the filtrate was made to 100ml with 80% acetone. Absorbance reading done at the 645nm wavelength respectively with 80% acid used as a blank.[12]
Phytochemical analysis

Determination of total phenolics

The concentration of total phenolics was examined spectrophotometrically employing the Folin–Ciocalteu reagent protocol as reported by.[13] In this assay, dry mass of leaves extract (50mg) was mingled first with 0.5ml of Folin-Ciocalteu reagent and then with 7.5ml of deionized water. The whole mixture was placed at room temperature for about 10min. After this, 1.5ml of 20% sodium carbonate was also added. The mixture was then heated in a water bath at 40°C for 20min and then it was cooled in an ice bath. At the end, the absorbance was read at 755nm with a spectrophotometer. Amount of TP was calculated by means of designing a calibration curve of gallic acid. The TPC results were shown with reference to gallic acid equivalents (GAE) per dry matter.

Determination of total flavonoids

Total flavonoid was conducted according to.[14] using aluminium chloride colorimetric method. 1ml of extracts was added with 4ml of distilled water in a flask. Then, 0.3ml of 5% NaNO2 was added. After 5min, 0.3ml of 10% AlCl3 was added and after 6min, 2ml of 1M NaOH was added. The mixture was diluted to 10ml with distilled water. The absorbance of the solution was measured at 510nm using a spectrophotometer. The results were expressed as mg catechin equivalents (CE)/g samples.

Determination of total tannins

Tannin content of the given sample was estimated by following the standard procedure.[15] The sample extract (1 ml) was mixed with Folin-Ciocalteau’s reagent (0.5 ml), followed by the addition of saturated Na2CO3 solution (1 ml) and distilled water (8 ml). The reaction mixture was allowed to stand for 30 min at room temperature. The supernatant was obtained by centrifugation and absorbance was recorded at 725 nm using UV-Visible Spectrophotometer. Increasing concentrations of standard tannic acid was prepared and the absorbance of various tannic acid concentrations was plotted for a standard graph. The tannin content was expressed as mg tannic acid equivalent per 100 gram of the sample.

Determination of total alkaloid content

The total alkaloid content was determined according to UV Spectrophotometer method.[16] This method is based on the reaction between alkaloid and bromocresol green. The part of the plant extract was dissolved in 2N HCl and then filtered. 1ml of this solution was transferred to separatory funnel and washed with 10ml chloroform The pH of phosphate
buffer solution was adjusted to neutral with 0.1N NaOH. One ml of this solution was transferred to a separating funnel and then 5ml of bromocresol solution along with 5ml of phosphate buffer were added. The mixture was shaken and the complex formed was fractioned with chloroform by vigorous shaking. The fractions were collected in a 10ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470nm. The results were averaged and reported in the form of mean ± S.E.M.

**Determination of saponin**

Saponin determine by the method of.[17] 20g of plant powder was put into a conical flask and 100ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90°C. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant.

**In vitro antioxidant assay**

**DPPH radical scavenging assay**

The free radical scavenging activity was measured by using the method of.[18] Briefly, 0.1mM solution of DPPH• in ethanol was prepared and 1ml of this solution was added to 3ml of leaf extract. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH free radical scavenging activity was calculated using the following formula

\[
\% \text{ scavenging} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}}\right] \times 100
\]

**Reducing power assay**

The reducing power of leaf of *Solanum nigrum* was determined according to the method of[19] 1ml of methanol leaf extract was dissolved in 1ml of distilled water, phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of TCA (10%) was added to the mixture,
which was then centrifuged for 10 min at 1000 × g . The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

RESULTS AND DISCUSSION

Height, Leaf area and Leaf length

Table-1 show the effect of cattle dung, vegetable waste compost and NPK fertilizer on the performance of Solanum nigrum. At week 6 after sowing, the application of vegetable waste and cattle dung produced the greatest plant height (38±3.16 cm) while the lowest plant height was obtained from the NPK treatment (36±3.14 cm) (Fig-1).

This results is in agreements with previous reports,[20] reported that plant height of Okra was greater in poultry dropping treated soil. They attributed it to the ready availability of nutrients for the easy absorption by plant root, thus resulting in an increase in plant growth.[21] Asserted that organic manuare treated soil could increase plant height when compared with other sources of fertilizer. Comparatively high nitrogen content of poultry manure buttresses the vegetative growth of crops.[22] Reported a positive effect of organic fertilizer on vegetative growth. The plants treated with NPK fertilizer grew fastest in the first three weeks. The plants treated with organic fertilizer grew higher from the sixth week up to the end of the experiment in week 10.

Table -1 showed the effect of organic (vegetable waste and cattle dung) and inorganic (NPK) fertilizer on leaf area and leaf length. The greatest leaf area and leaf length was observed in the organic fertilizer treatment (10.5±2.18cm & 5.5±1.58cm) and lowest in the inorganic fertilizer treatment (8.0±1.12cm & 4.0±1.48cm). Inc eased leaf area and leaf length implies higher light interception and dry matter product which invariably promotes plant growth.[23]

Table 1: Effect of organic and inorganic fertilizer on morphological traits of Solanum nigrum

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters (cm)</th>
<th>Organic fertilizer</th>
<th>Inorganic fertilizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Plant height</td>
<td>38±3.16</td>
<td>36±3.14</td>
</tr>
<tr>
<td>2</td>
<td>Leaf length</td>
<td>10.5±2.18</td>
<td>8±1.12</td>
</tr>
<tr>
<td>3</td>
<td>Surface area</td>
<td>5.5±1.58</td>
<td>4±1.48</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation
Fig 1: Effect of organic and inorganic fertilizer morphological traits of *Solanum nigrum*

The response of *Solanum nigrum* to organic manure application may also be attributed to the increasing total organic matter, macro and micronutrients rendered after the application of manure.

**Nutritional status, Phytochemicals & *In vitro* antioxidant activity**

Results in Table-2 indicate the effect of cattle dung, vegetable waste manure and NPK fertilizer on nutritional content of *Solanum nigrum*. Organic manure produced higher effect on carbohydrate, protein, amino acid, chlorophyll, vitamin c and chlorophyll content in the leaf of *Solanum nigrum* when compared with NPK fertilizer. Mean values recorded for the above parameters in leaf sample were 30.0±2.41, 1.93±1.93, 3.31±0.65, 2.53±0.72, 1.24±0.53mg/100g respectively compared with values of 10.0±2.26, 1.25±1.62, 2.0±0.52, 2.26±0.80, 1.98±0.40mg/100g respectively for inorganic fertilizer (Fig-2).

The result of comparative effect of organic and inorganic fertilizer on phytochemicals of *Solanum nigrum* was given in Table-3. Effect of organic fertilizer in plant phytochemical was higher than that of inorganic fertilizer. Values obtained for alkaloids, flavonoids, tannins, saponins and total phenol in organic treated leaf samples were 0.21±0.80, 0.9±0.53, 0.52±0.16, 0.34±0.36, 0.26±0.18mg/100g, respectively as against values of 0.2±0.32, 0.5±0.46, 0.4±0.12, 0.29±0.26, 0.8±0.14mg/100g for inorganic fertilizer.

Effect of organic and inorganic fertilizer on antioxidants of *Solanum nigrum* was shown in Table-4. Results revealed a similar trend of higher mean values for organic fertilizer when compared with inorganic fertilizer. Mean values recorded for leaf sample in organic fertilizer were 64.21±15.81, 35.42±12.80mg/100g respectively. Corresponding mean values for inorganic leaf sample were 42.12±14.72, 25.42±7.90mg/100g.
Preceding results are in harmony with those obtained by\cite{24} on African marigold (Tagetes erecta) who cited that, compost treatment increased chlorophyll a and b, carotenoids content, total carbohydrate. Also,\cite{25} on rosemary plants,\cite{26} on Montana plants found that, compost treatment increased plant pigments and total carbohydrate over NPK treatment plants and\cite{27} on tomato and cucumber found that, application of compost resulted in an increment over control plants. Hereabout\cite{28} on Barley and Maize and\cite{29} on Tobacco mentioned that, application of organic manuare increased photochemical activity of chloroplasts, total chlorophyll content as well as total carbohydrate.

This study on comparative effect of organic and inorganic fertilizers on nutritional analysis, phytochemicals, in vitro antioxidant level of Solanum nigrum revealed that organic fertilizer produced higher effects on all the parameters investigated when compared with inorganic fertilizer. The results of this study are in agreement with\cite{30-37} who have reported increases with organic fertilizers on phytochemical, nutritional and in vitro antioxidant. These increases could be due to the ease with which nutrients such as N, P and K in NPK fertilizers are lost by leaching. Nutrients in organic material are less easily available since the materials have to be decomposed and organic nutrients mineralized.\cite{38} Organic manures activate many species of living organisms which release phytohormones and may stimulate the plant growth and nutrients\cite{39} and such organisms need nitrogen for multiplication.\cite{40} The positive correlation shown in Table-3 between total phenolic and flavonoid compounds indicates that an increase in phenolic was followed by an increase in total flavonoid. Both were found to be highly correlated with antioxidant activity. Among Medan and Sri Pontian, it was found that Medan had higher total phenolics and total flavonoids. Phenolic compounds and flavonoids in this condition may play an important role as scavengers for free radicals and other oxidative species.\cite{41} Phenolic molecule is characteristic of a plant species or even of a particular organ or tissue of the plant.\cite{42}

Results of this study are also in consonance with results of the biggest and most extensive scientific study and research into the benefits of organic food by,\cite{43} who reported that organic food is more nutritious than non organic (ordinary produce) food and may in fact lengthen peoples lives. She also found that they contain higher levels of antioxidants and flavonoids which help ward off heart disease and cancer as well as iron and zinc. Research that was carried out in the Newcastle University also showed that organic food contain more antioxidants and less unhealthy fatty acid\cite{44} This could be attributed to the fact that the
nutrients in the organic manure are released gradually through the process of mineralization maintaining optimal soil levels over prolonged periods of time. Some of the organic substances released during the mineralization may act as chelates that help in the absorption of iron and other micro-nutrients.

**Table 2: Effect of organic and inorganic fertilizer on nutritional profile of *Solanum nigrum***

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameter</th>
<th>Organic Fertilizer</th>
<th>Inorgani Fertilizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrate</td>
<td>30.0±2.41</td>
<td>10.0±2.26</td>
</tr>
<tr>
<td>2</td>
<td>Protein</td>
<td>1.93±1.93</td>
<td>1.25±1.62</td>
</tr>
<tr>
<td>3</td>
<td>Amino acid</td>
<td>3.31±0.65</td>
<td>2.0±0.52</td>
</tr>
<tr>
<td>4</td>
<td>Chlorophyll</td>
<td>2.53±0.72</td>
<td>2.26±0.80</td>
</tr>
<tr>
<td>5</td>
<td>Ascorbic acid</td>
<td>1.24±0.53</td>
<td>1.98±0.40</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation

**Fig 2: Effect of organic and inorganic fertilizer on nutritional profile of *solanum nigrum***

**Table 3: Effect of organic and inorganic fertilizer on phytochemicals of *Solanum nigrum***

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter</th>
<th>Organic Fertilizer</th>
<th>Inorgani Fertilizer</th>
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<tr>
<td>1</td>
<td>Alkaloids</td>
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<td>0.2±0.32</td>
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<tr>
<td>2</td>
<td>Flavonoids</td>
<td>0.9±0.53</td>
<td>0.5±0.46</td>
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<tr>
<td>3</td>
<td>Tannins</td>
<td>0.52±0.16</td>
<td>0.4±0.12</td>
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<tr>
<td>4</td>
<td>Saponins</td>
<td>0.34±0.36</td>
<td>0.29±0.26</td>
</tr>
<tr>
<td>5</td>
<td>Total phenol</td>
<td>0.26±0.18</td>
<td>0.8±0.14</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation
Fig 3: Effect of organic and inorganic fertilizer on Phytochemical of *Solanum nigrum*

Table: 4. Effect of organic and inorganic fertilizer on in vitro antioxidant of *Solanum nigrum*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Samples</th>
<th>DPPH</th>
<th>Reducing power assay</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Organic fertilizer</td>
<td>64.21±15.81</td>
<td>42.12±14.72</td>
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<tr>
<td>2</td>
<td>Inorganic fertilizer</td>
<td>35.42±12.80</td>
<td>25.42±7.90</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation

Fig 4: Effect of organic and inorganic fertilizer on in vitro antioxidant of *Solanum nigrum*

CONCLUSION

Fertilisation is the most important and controllable factor affecting nutritional value of plants. The result indicate that application of cattle dung manure and vegetable waste compost can enhance the growth parameter, nutritional content, phytochemicals and antioxidant activity of *Solanum nigrum*. The effects of composts on plants are not solely attributed to the quality of mineral nutrition is provided but also to its other growth regulating components. Furthermore, the application of compost in the field enhances the quality of soils by increasing microbial activity and microbial biomass which are key components in nutrients cyclings, production of
plant growth regulators. Therefore, it could be concluded that, the chemical fertilizers of NPK could be replaced by the compost for improving the quality of the produced yield under safe agriculture conditions, in addition to decreasing the production costs and environmental pollution.

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REFERENCE


