Effect of Vermicompost and FYM on Pharmacological Activities of Cannabis sativa

Aloka Kumari
Research Centre for Plant Growth and Development, School of Life Sciences,
University of KwaZulu-Natal, Pietermaritzburg, Scottsville 3209, South Africa
Email: aloka.psrc@gmail.com

Abstract – Pharmacological activities of Cannabis sativa (Marijuana) include physiologic and metabolic restoration, antiatherosclerotic, antiaging, nerve tonic, cognitive function improvement in geriatric states and recovery from neurogenerative disorders like convulsions and tardive dyskinesia. The experiment was laid out in a Randomized Block Design (RBD) with two parameters (Vermicompost and Farmyard Manure) replicated thrice in different harvesting period. The data were collected for morphological traits, chlorophyll, free sugar, reducing sugar (Water soluble carbohydrates), protein, phosphorus, alkaloids and Dry Matter (DM) yield. Vermicompost 1.35 t/ha (Cow dung + Vegetable wastes + Eisenia fetida) recorded double yields as compared with FYM at 6.5 ton/ha. Approximately, Vermicompost showed three times more free sugar and reducing sugar content after 60 days. Determination of the total proteins in Cannabis sativa has revealed the tendency to their accumulation in Vermicompost, compared with FYM. The highest alkaloid present observed in leaf while the root and stem also were acceptable. The plant growth results indicate the presence of some growth-promoting substances in worm-processed material (Vermicompost). The Vermicompost also contained a considerable amount of some essential plant micronutrients that might be responsible for better plant growth and productivity. This study suggests that vermicomposted manures may be a potential source of plant nutrients for sustainable crop production.

Keywords – Cannabis sativa, Farmyard Manure, Vermicompost.

I. INTRODUCTION

Marijuana (Cannabis sativa L.) is the most abundant illegal drug of abuse in the United States, and although authorities seized more than 1200 metric tons in 2001, marijuana trafficking today is a thriving, multibillion dollar industry. Drug enforcement requires forensic tools that provide evidence for conspiracy in the cultivation and distribution of marijuana. The capacity to pinpoint the geographic origins of seized drugs and to identify Cannabis cultivars has further forensic utility. Cannabis is one of the oldest known domesticated plants and today is cultivated throughout the world for psychoactive cannabinoids, durable fiber, and nutritious seed. An estimated survey indicated that the use of herbal medicine will reach to the tune of three trillion USD during 2050. Currently, WHO encourages, recommends and promotes the inclusion of herbal drugs in national health care programs.

Among the sources of organic manures, Vermicompost has a special place because of the presence of readily available plant nutrients, growth enhancing substances, and number of beneficial microorganisms like N2 fixing, P solubilising and cellulose decomposing organisms [1]. Such drugs are easily available at a reasonable price within the reach of common man and as such are time tested and thus considered to be much safer than the modern synthetic drugs. Although the main psychoactive chemical compound in Cannabis sativa is Δ2-tetrahydrocannabinol (THC), the plant is known to contain about sixty cannabinoids; however, the most of these "minor" cannabinoids are only produced in trace amounts. Besides THC, another cannabinoid produced in high concentrations by some plants is cannabidiol (CBD), which is not psychoactive but has recently been shown to block the effect of THC in the nervous system [2]. Differences in the chemical composition of marijuana varieties may produce different effects in humans. Synthetic THC, called dronabinol, does not contain CBD, CBN, or other cannabinoids, which is one reason why its pharmacological effects may differ significantly from those of natural marijuana preparations. The root is remembered by some as an old folk remedy for arthritis or joint pain [3] and references were also found stating the roots emetic and cathartic properties. The roots are the least studied part of the cannabis plant; still, several components and compounds were identified since the 1970’s. Although glandular hairs are where the majority of cannabinoids are produced they have also been detected in the roots by immunoassays [4] and chemical analysis [5]. Terpenes have been detected and isolated from essential oil from flowers, leaves and roots [6]. The terpenes are responsible for the flavor of different varieties of cannabis and determine the preference of the cannabis users [7]. Alkaloids are another class of chemical constituents that have been found in cannabis and both piperidine and pyrrolidine were identified and isolated from the roots, leaves, stems, pollen, and seeds [6]. Cannabis fruits and roots have yielded 11 compounds identified as phenolic amides and lignanamides [8]. A review done by [7] found phenolic amides to have cytotoxic, anti-inflammatory, antineoplastic, cardiovascular and mild analgesic activity and the lignans to have insecticidal effects.

Decomposition of various organic substrates (kitchen waste, agro-residues, institutional and industrial wastes including textile industry sludge and fbers) into valuable vermicompost has been extensively studied using an exotic earthworm species (epigeic- Eisenia fetida) [9] - [11]. Tests have also been conducted combining thermo-composting and vermicomposting to improve efficiency and compost quality [11] - [13]. Khalili [13] advocated the integrated use of organic and inorganic nutrient sources with effective microorganisms (EM) for improving cotton
yield. The effects of earthworm-processed sheep-manure (vermicompost) on the growth productivity and chemical characteristics of soybean straw (Glycine max L. Merril.), wheat straw (Triticum aestivum L.), maize stover (Zea mays L.), chickpea straw (Cicerarietinum L.), city garbage [14] and greenhouse tomatoes (Lycopersicum esculentum) [15] has also been studied. Earthworm species such as *Eudrilus eugeniae* are voracious feeders of organic wastes, and their presence has been found to reduce the time required for composting [16]. Significantly higher protein was observed under treatment of organic manure viz. 10 ton/ha with spacing 30 X 45 cm and its morphological study and its biogeochemical study have been done. Difference between treated and untreated plants has been studied.

The objectives of this project were: 1) To determine the optimal vermicompost/soil admixture for growth of *Cannabis sativa*; and 2) To compare the growth of geraniums in the optimal vermicompost/soil admixture to those grown in standard potting soil supplemented with chemical fertilizer.

## II. MATERIAL AND METHODS

### A. Plant Collection

Wild variety seeds of *Cannabis sativa* were collected from Banka district 40 km away from the study area and planted in University Department of Botany, T. M. Bhagalpur University dried for a week at room temperature (25±2 C) and stored in screw capped bottles under ambient condition before experiment. Best quality of seeds has been selected by germination test [17] and Tetrazolium test [18]. Plants were transplanted after three weeks in 30 x 45 cm spacing in field. Experiment was conducted in Post Graduate Botany Department, T.M, Bhagalpur University, Bhagalpur for two years. At the initial stage of flowering and fruiting when active constituents present in high level plants collected from field for morphological and biogeochemical analysis have been experimented.

### B. Morphological and Physical Analysis of Plants

On the basis of best quality physical and chemical profiles of FYM and Vermicompost (Cow dung + Vegetable wastes + *Eisenia foetida*) have been selected for field treatment. Following data were taken 25 plants were taken for each study.

\[
T_1 = \text{FYM at 6.5 ton/ha.}
\]
\[
T_2 = \text{Vermicompost at 1.35 ton/ha. (Cow dung + Vegetable wastes + *Eisenia foetida*)}
\]

#### a. Root Length

The experimental plants were uprooted and washed in running water properly. The root lengths were estimated before root samples were stained. The washed roots were cut into 1cm bits in a petridish containing water. Aliquots of root bits were then taken in a square grid (1cm). Petridish and the number of intersects i.e. points where root bits intersect the grids were counted. The same process was used for the entire root sample.

The total root length was estimated by formula:

\[
\text{Total root length (cm)} = \frac{N \times 14}{X \times \text{grid size}}
\]

Where, \(N=\) Number of intersects; \(11/14 =\) Tennant’s factor

#### b. Radius of Root

The root radius was estimated by slide Caliper. The diameter was measured; five readings were taken which include the minimum as well as maximum diameter region.

#### c. Surface Area of Root

The surface area of root was derived by using the general formula:

\[
\text{Surface area of Root}=2\pi r
\]

Where, \((\pi=3.14, r=\text{radius of root, } l=\text{length of root})\)

#### d. Root Volume

Plants were uprooted and roots of the sample plants were washed properly in running tap water without loss of any branch. The washed roots were placed properly on the blotting paper to remove water molecules of surface. The volume of root was taken by immersing the entire root in measuring cylinder full with water. Root volume was represented by ml³.

#### e. Shoot length

The shoot length of plant was measured by ruler scale. Length of the branches and basal stem length which showed highest length were taken into account. It was derived by following formula:

\[
\text{Mean shoot length} = \frac{\text{Length of basal stem} + \frac{\text{Total length of all branches of plant}}{\text{Total number of branches taken}}}{2}
\]

#### f. Radius of stem

The surface area of shoot was derived by using general formula:

\[
\text{Surface area of shoot} = 2\pi rl
\]

Where, \((\pi=3.14, r=\text{radius of root, } l=\text{length of root})\)

#### g. Area of leaf

Leaf was measured by using Graph paper method. The leaves were clipped and marked on graph paper (10 x 10 mm).

Mean leaf area

\[
= \frac{\text{Sum total no. of leaves of all the repli}}{\text{Total no. of leaf of plant}}
\]

#### h. Number of leaves

The total number of leaves of an individual plant was derived by using general formula:

\[
\text{Mean number of leaves} = \frac{\text{Sum total no. of leaves of all the replicates}}{\text{Number of replicates}}
\]

#### i. Dry matter (DM) yield

The complete plants were picked up at the first and third harvests at 50% of flowering. They were dried in the open-air area after separation of leaf, stem and root. One sample of which was oven dried at the temperature of 100°C after weighing and dry matter (DM) yield was calculated based on gr/m².

### C. Estimation of Chl a and Chl b, chlorophyll

The estimation of total chlorophyll content was assisted by colorimetric method [19] with some modifications. 50 mg of fresh leaf sample was weighed and placed into mortar and then crushed thoroughly with pestle. Ten mL of 80% acetone was added to allow the tissue to be thoroughly homogenized and centrifuged for 5 min at
For Vermicompost, the dried plant parts were crushed subsequently, and the reaction was found to be stable.

**3.3 Estimation of total sugar and reducing sugar**

For LPO estimation one gram of samples were ground in a mortar and pestle in 5 mL of 80% ethanol (v/v) and the mixture was boiled for 10 min and centrifuged at 2000 rpm for 10 min, the supernatant was collected and the pellet was re-extracted in 5 mL of hot 80% ethanol. Supernatants from both extractions were combined, and total soluble sugar, reducing sugars and Water soluble carbohydrates (WSC) were then determined by the phenol sulfuric acid [20].

**4. Estimation of Protein**

The method of [21] was used for protein estimation. Bovine serum albumin (BSA) powder was dissolved in distilled water and diluted to a concentration of 1 µg/µL. A series of dilutions (0, 1, 2.5, 5, 10, and 20 µg/µL) were made in replicates of 4 with a final volume of 100 µL. Samples were diluted such that they would fall within the BSA standard range (0-25 µg / 100 µL) and 100 µL placed in each well. After standards and samples were diluted and transferred to the microplate, 200 µL of biuret reagent was added to each well and mixed thoroughly with repeated pipetting. Biuret reagent was prepared by mixing 0.5 ml of 1% cupric sulfate with 0.5 ml of 2% sodium potassium tartrate, followed by the addition of 50 ml of 2% sodium carbonate in 0.1 N NaOH. The mixture was then allowed to incubate at room temperature for 10-15 minutes prior to the addition of 20 µL per well of 1.0 N Folin & Ciocalteu’s reagent. Samples were mixed immediately with repeated pipetting with each addition. Colour was allowed to develop for 30 minutes at room temperature and the absorbance measured at 650 nm and blanked on the water only control. Although in these experiments the plates were read immediately, the reaction was found to be stable for up to an hour.

**5. Estimation of Phosphorus**

A wide range of microbial P solubilization mechanisms exist in nature and much of the global cycling of insoluble organic and inorganic soil phosphates is attributed to bacteria and fungi [22].

**6. Estimation of Alkaloid**

Quantitative analysis of alkaloid was conducted by the [23]. The harvested plant parts were washed thoroughly with distilled water and dried at room temperature for 48 hours. The dried plant parts were crushed subsequently, powdered and kept in glass container for experiment. 10gm of powdered sample was soaked in 28% ammonium hydroxide solution and little dried up. Subsequently the sample was soxhletted with a mixture of chloroform and ethanol (3:1v/v) for 8 hours. 100 mL of solvent was extracted and vigorously shaken with 25ml of N/2 H2SO4 and the acid extract was collected. The process was repeated thrice for the complete extraction of alkaloids. The combined acid extract from alkaline extract with 20 and 15ml of chloroform. The chloroform extract was distilled on water bath until only few ml were left. The left solvent was completely dried up and left residue was weighed on monopan balance to calculate the total crude alkaloids.

**H. Statistical**

In this study, different treatments with three replications were analyzed, though data statistically were analyzed by complete Randomized design. Experiment SAS program and means were compared with Duncan at 1% and 5% level.

**III. Result**

The results of mean comparison in two physical and chemical profiles of FYM and Vermicompost are shown in Tables 1 and 9. Effect of Vermicompost and FYM for trait of root and shoot lengths were significant at 1% level after 60 days. Although the average of root and shoot radius in Vermicompost were more than FYM but effect of these two different growth conditions were not significant after 30 and 60 days. In comparing the average root volume of 10 samples of Cannabis sativa, Vermicompost with the average of 1.68 ml and 2.62 ml3 had higher capacity after 45 and 60 days, respectively. Comparison of means in surface area of shoot and root treatments indicated that Vermicompost has more effective role during of 60 days. These results show that plant cell size and mitosis activity are considerable in Vermicompost and Cannabis sativa may be resist to dry tension in this growth condition more than farm (Table 1).

According to Table 2, the Duncan test results showed that the mean differences between two growth conditions were no significant in number of leaves and pinnula area after 45 days but Vermicompost had significant result for pinnula area after 60 days with 242.29 cm2. There were significant differences between Vermicompost and FYM for DM yield during 60 days. Weight of separated parts of plant like leaf, stem and root were higher in Vermicompost which were 0.58, 0.71 and 0.18 gm/cm², respectively (Table 3). The global chlorophyll seasonal patterns of highs and lows are generally consistent among these datasets, although some differences occur (table 4). For Vermicompost data, the maximum chlorophyll concentrations were observed after 60 days when the total chlorophyll was 3.365 (µg/mg). There were significant differences between these two conditions for different types of chlorophyll while there was no difference in total chlorophyll. Free sugar and reducing sugar content were assisted in Table 5 and 6. After the statistical processing of data, the obtained results indicated substantial differences in reducing sugar and free sugar content between Vermicompost and FYM either plant different part (leaf, stem and root) or complete plant. Approximately, Vermicompost showed three times more free sugar and reducing sugar content based on mg per plant after 60 days. The most difference between these two growth conditions was in root samples in free sugar and reducing content.
Determination of the total proteins in *Cannabis sativa* has revealed the tendency to their accumulation in Vermicompost, compared with FYM. Specific peculiarities were revealed both in protein content and ability of their accumulation. This index in leaves of Vermicompost plants were much higher compared with FYM. The highest content of proteins in different part of plant (leaf, stem and root) was detected in leaves after 60 days (Table 7).

Whole plant after Vermicompost and FYM treatment recorded 0.24 mg/plant and 0.049 mg/plant after 60 days, respectively. Significant differences also were observed in leaf, stem and root after different harvesting period (Table 8). Result of alkaloid showed 1.135 mg/plant in Vermicompost treatment while in FYM recorded about 0.139 mg/plant. This might be due to the better availability of nutrients from organic and foliar source of nutrients and effective conservation of nutrients such as Fe, Mg and Zn at site of photosynthesis into pigments. The highest alkaloid present observed in leaf while the root and stem also were acceptable (Table 9).

**IV. DISCUSSION**

These experiments, together with others reported in the literature, demonstrate that Vermicomposts have considerable potential for improving plant growth significantly, when used as components of horticultural soil or container media. Nevertheless, there appear to be major differences between the effects of the Vermicomposts and FYM that were used in our study, in terms of their influence on plant growth, depending upon the source of the parent waste material used in their production. These differences in growth responses could be due in part to fundamental differences between the FYM and Vermicomposting processes which use quite different microbial communities, with composting tending to result in the release of mineral nitrogen in the ammonium form, whereas vermicomposting releases most of the nitrogen in the nitrate form [24], the form readily available for plant uptake. The high demands for chemical fertilizer meets nutrients whereas organic manure initially form conducive environment with regard to physical parameters of soil which promote better root growth and other vegetative growth. It is assured that other factors, such as the presence of beneficial microorganisms or biologically active plant growth influencing substances such as phytohormone are released by beneficial microorganisms present in the Vermicompost rich soil [24], [25]. Root initiation, increased root biomass, enhanced plant growth and development and sometimes, altera-tions in plant morphology are among the most frequently claimed effects of Vermicompost treatment [26]. Plant and crop physiologists, microbiologists and agronomists agree that plant growth and development are strictly dependent on biological fertility factors. Earthworms stimulate microbial activities and metabolism and also influence microbial populations. As a consequence more available nutrients and microbial metabolites are released into the soil [26].

The present study has created interesting data with respect to plant growth, yield characters and biochemical analysis. As evidenced from the results of [27] and [28] this may be due to effective micro-organism enhances the production of phytohormones like auxins and gibberellins that might have stimulated the growth by increasing the plant height, number of branches. Similarly, Neilson [29-30] and Tomati [31] have also reported that vermicompost contained growth promoting hormone auxins, cytokinins and flowering hormone gibberellins secreted by earthworms. Humic acid influences plant growth through modifying the physiology of plants and by improving the physical, chemical and biological properties of soil [32]. Humic acid provides carbon as an energy source to nitrogen fixing bacteria, thus proves its biological function. The natural bioregulator in *Moringa* leaf extract also increased the dry matter production registered increased yield compared to control. Plants, such as vegetables and fruits, have satisfactory edible proteins only if they are safe with high quality so that they can be used by humans [33]. Unlike other composts, Vermicompost also contains worm mucus which helps prevent nutrients from washing away, holds moisture better and thus helps in increased plant growth [34]. In present study *Cannabis sativa* have high protein in Vermicompost. Therefore, as this growth condition plants are rich in proteins, these can be utilized as non-conventional bio-nutritional sources.

**V. CONCLUSION**

Vermicomposts have the potential for improving plant growth when added to greenhouse container media or soil. The optimal plant growth in our study, which was conducted only over a short period of time, was in pots containing Cow dung, Vegetable wastes and *Eisenia fetida*. In present study, after expalnting the test plants, soil sample showed improved level of total P and alkaloid in the range over FYM sample. Vermicompost treatment had better result in protein, phosphate and sugar content thus, it is economic and easily applicable by nursery workers and poor farmers in developing mass planting stock over costly plant growth regulators and associated technical use in rapid multiplication.

**VI. ACKNOWLEDGEMENT**

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AUTHOR’S PROFILE

Dr. Aloka Kumari
F.S.Sc., F.S.A.B (Ph.D) is an innovative researcher at Research Centre for Plant Growth & Development, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa. She worked as a Women Scientist (Principal Investigator) under Department of Science and Technology; Government of India at University Department of Botany, T. M. Bhagalpur University, Bhagalpur. She has more than 9 years research experience in R & D and novel materials having application for Science and Society. She completed one major project and three minor projects and presently she is presently involved in six minor projects. She has published 2 books and 22 research articles in peer reviewed national and international journals in different fields of reproductive biology, molecular biology, tissue culture as well as pharmacological studies of medicinal plants. She has presented at more than 17 National and International Conferences/ Seminars. She is associated several journals viz. member of Editorial Board, Global Advanced Research Journal of Agricultural Science (GARJAS); Executive Editor and Coordinator (Sciencia: A multidisciplinary Journal). She has Society for Applied Biotechnology (F.S.Sc.), Society of Sciences (F.S.A.B). India. She has also organized several societal training programmes for farmers to promote Cultivation, propagation and marketing of medicinal plant for rural development in collaboration with many NGOs. viz. Akhand Deep Sansthan, Bhagalpur Zila Gram Vikas Sansthan, Safali Sansthan. She is member of Society of Plant Reproductive Biologists, Nutrition Society of India, Asian Journal of Chemistry, Botanical Society of India, Treasurer of Akhand Deep Sansthan (Registered Society).


Table 1: Effect of Vermicompost and FYM on Root and Shoot growth in Cannabis sativa

<table>
<thead>
<tr>
<th>Days</th>
<th>Length (cm)</th>
<th>Radius (cm/plant)</th>
<th>Volume (ml²)</th>
<th>Surface area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₁</td>
<td>T₂</td>
<td>T₁</td>
<td>T₂</td>
</tr>
<tr>
<td>30</td>
<td>7.34±0.61</td>
<td>9.35±0.86</td>
<td>0.06±0.02</td>
<td>0.06±0.02</td>
</tr>
<tr>
<td>45</td>
<td>9.9±0.42</td>
<td>11.02±0.5</td>
<td>0.07±0.02</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>60</td>
<td>10.44±1.0</td>
<td>16.82±0.43</td>
<td>0.15±0.09</td>
<td>0.21±0.06</td>
</tr>
</tbody>
</table>

**Root growth**

<table>
<thead>
<tr>
<th>Days</th>
<th>Surface area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>1.97±0.1</td>
</tr>
<tr>
<td>45</td>
<td>3.40±0.1</td>
</tr>
<tr>
<td>60</td>
<td>5.45±0.1</td>
</tr>
</tbody>
</table>

**Shoot growth**

<table>
<thead>
<tr>
<th>Days</th>
<th>Pinnula Area (cm²)</th>
<th>% increase over control</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.14±0.11</td>
<td>0.16±0.019</td>
</tr>
<tr>
<td>45</td>
<td>0.21±0.02</td>
<td>0.23±0.02</td>
</tr>
<tr>
<td>60</td>
<td>0.23±0.07</td>
<td>0.24±0.21</td>
</tr>
</tbody>
</table>

**,** *, ns: correlation coefficients between conditions were significant at 1% and 5% probability level, and non-significant, respectively.

The means with same small letters were not significantly different as per Duncan’s multi-range test at P<0.05.

Value of Mean ± SE of 10 samples; T₁= FYM; T₂= Vermicompost

Table 2: Effect of Vermicompost and FYM on Leaf growth in Cannabis sativa

<table>
<thead>
<tr>
<th>Days</th>
<th>No.of leaves/plant</th>
<th>% increase over control</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>6.68±0.42</td>
<td>37.18±2.56</td>
</tr>
<tr>
<td>45</td>
<td>9.67±0.91</td>
<td>10.67±0.67</td>
</tr>
<tr>
<td>60</td>
<td>14.84±1.17</td>
<td>17.01±1.16</td>
</tr>
</tbody>
</table>

**,** *, ns: correlation coefficients between conditions were significant at 1% and 5% probability level, and non-significant, respectively.

The means with same small letters were not significantly different as per Duncan’s multi-range test at P<0.05.

Value of Mean ± SE of 10 samples; T₁= FYM; T₂= Vermicompost

Table 3: Dry Matter (DM) yield of Cannabis sativa at different harvesting period

<table>
<thead>
<tr>
<th>Days</th>
<th>Leaf (gm/m²)</th>
<th>Stem (gm/m²)</th>
<th>Root (gm/m²)</th>
<th>Total dry matter(gm/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.18±0.13</td>
<td>0.21±0.06</td>
<td>0.18±0.10</td>
<td>0.21±0.01</td>
</tr>
<tr>
<td>45</td>
<td>0.29±0.01</td>
<td>0.36±0.03</td>
<td>0.23±0.14</td>
<td>0.46±0.01</td>
</tr>
<tr>
<td>60</td>
<td>0.38±0.01</td>
<td>0.58±0.01</td>
<td>0.33±0.19</td>
<td>0.71±0.04</td>
</tr>
</tbody>
</table>

**,** *, ns: correlation coefficients between conditions were significant at 1% and 5% probability level, and non-significant, respectively.

The means with same small letters were not significantly different as per Duncan’s multi-range test at P<0.05.

Value of Mean ± SE of 10 samples; T₁= FYM; T₂= Vermicompost

Table 4: Chlorophyll content at different harvesting period

<table>
<thead>
<tr>
<th>Days</th>
<th>Chlorophyll a (µg/mg)</th>
<th>Chlorophyll b (µg/mg)</th>
<th>Total Chlorophyll (µg/mg)</th>
<th>Chi a:Chl b (µg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>2.52±0.08</td>
<td>4.88±0.07</td>
<td>1.19±0.06</td>
<td>1.37±0.023</td>
</tr>
<tr>
<td>45</td>
<td>3.88±0.05</td>
<td>4.56±0.05</td>
<td>1.44±0.1</td>
<td>1.89±0.02</td>
</tr>
<tr>
<td>60</td>
<td>4.17±0.1</td>
<td>4.95±0.64</td>
<td>1.24±0.07</td>
<td>1.87±0.04</td>
</tr>
</tbody>
</table>

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The means with same small letters were not significantly different as per Duncan’s multi-range test at P<0.05.

Value of Mean ± SE of 10 samples; T₁= FYM; T₂= Vermicompost

Table 5: Free sugar content at different harvesting period

<table>
<thead>
<tr>
<th>Days</th>
<th>Leaf (µg/mg)</th>
<th>Stem (µg/mg)</th>
<th>Root (µg/mg)</th>
<th>Whole plant (mg/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>5.05±0.01</td>
<td>6.33±0.36</td>
<td>3.05±0.02</td>
<td>3.96±0.3</td>
</tr>
<tr>
<td>45</td>
<td>5.48±0.06</td>
<td>7.78±0.31</td>
<td>3.81±0.13</td>
<td>4.83±0.01</td>
</tr>
<tr>
<td>60</td>
<td>6.04±0.02</td>
<td>8.17±0.41</td>
<td>4.03±0.01</td>
<td>5.41±0.01</td>
</tr>
</tbody>
</table>

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The means with same small letters were not significantly different as per Duncan’s multi-range test at P<0.05.

Value of Mean ± SE of 10 samples; T₁= FYM; T₂= Vermicompost
Table 6: Reducing sugar content at different harvesting period

<table>
<thead>
<tr>
<th>Days</th>
<th>Leaf (µg/mg)</th>
<th>Stem (µg/mg)</th>
<th>Root (µg/mg)</th>
<th>Whole plant (mg/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>30</td>
<td>0.35±0.01</td>
<td>0.53±0.006</td>
<td>0.30±0.01</td>
<td>0.35±0.004</td>
</tr>
<tr>
<td></td>
<td>0.24±0.006</td>
<td>0.41±0.001</td>
<td>0.15±0.01</td>
<td>0.23±0.005</td>
</tr>
<tr>
<td>45</td>
<td>0.48±0.01</td>
<td>0.74±0.02</td>
<td>0.41±0.01</td>
<td>0.58±0.004</td>
</tr>
<tr>
<td></td>
<td>0.30±0.01</td>
<td>0.67±0.01</td>
<td>0.25±0.01</td>
<td>0.70±0.04</td>
</tr>
<tr>
<td>60</td>
<td>0.50±0.01</td>
<td>0.85±0.01</td>
<td>0.49±0.02</td>
<td>0.64±0.008</td>
</tr>
<tr>
<td></td>
<td>0.42±0.002</td>
<td>0.89±0.01</td>
<td>0.54±0.01</td>
<td>1.29±0.007</td>
</tr>
</tbody>
</table>

**+, *, ns : correlation coefficients between conditions were significant at 1% and 5% probability level, and non-significant, respectively.

The means with same small letters were not significantly different as per Duncan’s multi-range test at P<0.05.

Table 7: Changes in protein content at different harvesting period in Cannabis sativa.

<table>
<thead>
<tr>
<th>Days</th>
<th>Leaf (µg/mg)</th>
<th>Stem (µg/mg)</th>
<th>Root (µg/mg)</th>
<th>Whole Plant (mg/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>30</td>
<td>36.91±0.05</td>
<td>53.92±0.01</td>
<td>17.55±0.2</td>
<td>18.59±0.28</td>
</tr>
<tr>
<td></td>
<td>17.95±0.3</td>
<td>23.33±0.1</td>
<td>10.93±0.3</td>
<td>17.50±0.2</td>
</tr>
<tr>
<td>45</td>
<td>40.99±0.09</td>
<td>56.38±0.05</td>
<td>20.65±0.2</td>
<td>22.63±0.51</td>
</tr>
<tr>
<td></td>
<td>20.29±0.09</td>
<td>33.53±0.3</td>
<td>17.72±0.5</td>
<td>39.66±0.14</td>
</tr>
<tr>
<td>60</td>
<td>42.31±0.25</td>
<td>63.78±0.09</td>
<td>24.86±1.0</td>
<td>31.07±0.27</td>
</tr>
<tr>
<td></td>
<td>20.66±0.02</td>
<td>37.97±0.1</td>
<td>25.79±0.1</td>
<td>71.47±0.6</td>
</tr>
</tbody>
</table>

**+, *, ns : correlation coefficients between conditions were significant at 1% and 5% probability level, and non-significant, respectively.

The means with same small letters were not significantly different as per Duncan’s multi-range test at P<0.05.

Table 8: Phosphate content at different harvesting period

<table>
<thead>
<tr>
<th>Days</th>
<th>Leaf (µg/mg)</th>
<th>Stem (µg/mg)</th>
<th>Root (µg/mg)</th>
<th>Whole plant (mg/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>30</td>
<td>0.051±0.02</td>
<td>0.064±0.03</td>
<td>0.037±0.01</td>
<td>0.049±0.01</td>
</tr>
<tr>
<td></td>
<td>0.026±0.01</td>
<td>0.029±0.01</td>
<td>0.016±0.01</td>
<td>0.025±0.14</td>
</tr>
<tr>
<td>45</td>
<td>0.067±0.01</td>
<td>0.116±0.003</td>
<td>0.06±0.002</td>
<td>0.111±0.06</td>
</tr>
<tr>
<td></td>
<td>0.02±0.002</td>
<td>0.04±0.002</td>
<td>0.028±0.01</td>
<td>0.095±0.01</td>
</tr>
<tr>
<td>60</td>
<td>0.079±0.01</td>
<td>0.19±0.005</td>
<td>0.059±0.01</td>
<td>0.178±0.001</td>
</tr>
<tr>
<td></td>
<td>0.024±0.01</td>
<td>0.061±0.01</td>
<td>0.049±0.03</td>
<td>0.24±0.13</td>
</tr>
</tbody>
</table>

**+, *, ns : correlation coefficients between conditions were significant at 1% and 5% probability level, and non-significant, respectively.

The means with same small letters were not significantly different as per Duncan’s multi-range test at P<0.05.

Table 9: Presence and Alkaloids content at harvesting period in fresh and dry material

<table>
<thead>
<tr>
<th>Alkaloid content (mg/plant)</th>
<th>% of dry matter (mg/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>Stem</td>
</tr>
<tr>
<td>0.738±0.051</td>
<td>1.135±0.15</td>
</tr>
</tbody>
</table>

(- = Absent; + = Moderately presence; ++ = Good presence; +++ = Excellent present)

**+, *, ns : correlation coefficients between conditions were significant at 1% and 5% probability level, and non-significant, respectively.

Value of Mean ± SE of 10 samples; T1 = FYM; T2 = Vermicompost