Effect of Vermicompost on Useable Biomass Yield, Cannabinoids and Terpenes Content of Indoor Grown *Cannabis sativa* L. Plants

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ABSTRACT

The effect of application of vermicompost on useable biomass yield, cannabinoids and terpenes content on *Cannabis sativa* L. plants was evaluated. Vegetative cuttings taken from a screened and selected mother plant were grown in biodegradable jiffy pots for rooting. Well rooted plants were transplanted in 5 gallons pots for vegetative growth (18 hours photoperiod). After a desirable vegetative growth, plants were subjected to flowering (12 hours photoperiod). From the beginning plants were divided in two groups, (1) control and (2) treated with vermicompost liquid extract till maturity. Plants of both groups were kept in the same climatic control environment and, were watered and fertilized normally. At maturity, both groups of plants were harvested and processed for usable dry biomass. Plants of both groups were compared for biomass production per plant, cannabinoids and terpenes content. Our results show that plants treated with vermicompost liquid extract have produced about 15% higher biomass and about 30% higher yield of THC per plant as compared to those with the regular fertilizer. There was no difference noted in the terpenes content of the control vs treated plants.

Key Words: Biomass yield, *Cannabis sativa* L., Cannabidiol, Δ^9- Tetrahydrocannabinol, Terpenes

INTRODUCTION

Taxonomically, cannabis is a single but highly variable species, *Cannabis sativa* L. (Small and Cronquist, 1976; Small 2015). It is an annual and dioceous but occasionally monoecious plant. Also, cannabis is a wind pollinated plant which is highly allogamous in nature. It is widely distributed in nature and can be found in all kinds of habitats from tropics to foot hills of alpines. Cannabis is cultivated for millennia for the use of grain, fiber as well as for recreational, medical, and ritual purposes. Traditionally, the plant has been used for the treatment of a variety of ailments such as headache, asthma, diarrhea, constipation, pain and anxiety, just to name a few, since ancient times in different forms.

Cannabis has been reported to contain more than 560 different compounds (ElSohly et al. 2017) belonging to a diverse group of chemical classes, the most important of which is the cannabinoids. There are 120 cannabinoids reported so far (ElSohly et al. 2017), among which Δ^9-Tetrahydrocannabinol (Δ^9-THC) and Cannabidiol (CBD) are the two major natural cannabinoids having very different pharmacological profiles with a tremendous therapeutic potential. It accumulates mainly in the glandular trichomes of the plant (Hammond and Mahlberg 1977). The structure of THC was determined by Gaoni and Mechoulam (1964). THC possesses analgesic, anti-inflammatory, appetite stimulant, and antiemetic properties making it a very promising therapeutic agent, especially for cancer and AIDS patients (Sirikantaramas et al. 2007). Many reports are available citing the pharmacologic and therapeutic potency of preparations of cannabis and its main active constituent Δ^9-THC (Mechoulam1986; Grinspoon and Bakalar 1993; Mattes et al. 1994; Brenneisen et al. 1996; Pryce and Baker 2005; Abrams et al. 2007; Cascio et al. 2017). Beside THC, cannabidiol (CBD) is another important cannabinoid which is non-psychoactive and highlighted for its activity against childhood epilepsy syndromes and other disorders. CBD was first isolated from Mexican marijuana (Adams et al. 1940) and the structure was determined by Mechoulam and Shvo in 1963 (Mechoulam and Shvo, 1963). Other than THC and CBD, major cannabinoids found in cannabis are

tetrahydrocannabivarin (THCV), cannabinol (CBN), cannabigerol (CBG) and cannabichromene (CBC). Based on its chemical profile cannabis can be categorized in two distinct classes (a) drug type variety with high cannabinoids content and (b) fiber type variety (with THC < 0.30%). Among the drug type, it can be divided in three different varieties (1) high THC variety, (2) high CBD variety and (3) intermediate variety (THC~CBD).

Beside cannabinoids, terpenes or isoprenoids, consist of the second largest class of cannabis constituents. These compounds are responsible for the characteristic aroma of the plant. Terpenes can be classified into five main classes: monoterpenes, sesquiterpenes, diterpenes, triterpenes, and miscellaneous terpenes. A total of 120 terpenes in cannabis are reported so far (Radwan et al. 2021). Out of 120 terpenes, there are 61 monoterpenes (C_{10} skeleton), 51 sesquiterpenes (C_{15} skeleton), 2 diterpenes, (C_{20} skeleton), 2 triterpenes (C_{30} skeleton), and 4 miscellaneous compounds. Only a few reports have been published on the possible contribution of terpenes to the activity of cannabis. For example, pinene has been reported as an acetylcholinesterase inhibitor aiding memory, which may counteract THC intoxication side effects (Miyazawa and Yamafuji, 2005 and Nissen et al. 2010). The sesquiterpene β-caryophyllene (reaching 2 mg/g), the most predominant sesquiterpene found in cannabis, was shown to interact with cannabinoid receptor type 2, and be responsible for the anti-inflammatory effects of some cannabis preparations (Gertsch et al., 2008 and Klauke et al. 2014). Interestingly, caryophyllene oxide has been reported as the main component responsible for cannabis identification by drug-sniffing dogs (Russo, 2011). Much more research on cannabis terpenes' pharmacology, synergism, and mechanism of action is therefore needed to fully understand the contribution of terpenes in the activity of cannabis.

In light of medicinal properties and other uses of cannabis and its useful constituents, research is ongoing to increase the quality and productivity of this crop. In general, to increase the productivity of crops new methods such as use of modern synthetic

fertilizers, pesticides, genetically modified varieties of crop etc. has been used in last few decades. No doubt that these methods had a positive impact on crop production for the short term but resulted in lots of damage to soil health and plant productivity in general. Traces of these chemicals in the crop products may have unembellished negative effects on the health of consumers. Therefore, an interest is developed towards the organic fertilizers or vermicompost. vermicomposting is a sustainable technique for solid waste disposal and reported to increase the fertility and productivity of the land and produce nutritive and safe food (Ramesh et al., 2005). The objective of present study is to evaluate the effect of application of vermicompost on useable biomass yield, cannabinoids and terpene content on *Cannabis sativa* L. plants.

MATERIAL AND METHODS

Plant Material

Vegetative cuttings of *Cannabis sativa* L. were taken from a screened and selected high THC yielding elite mother plant. These cutting were kept under a similar climatic controlled condition for rooting and vegetative growth. Cuttings/clones were initiated in 4" biodegradable jiffy pots for rooting. Well rooted plants were transplanted to regular grow pots (size: height 11" and dimeter 12" on top) and were kept under vegetative light cycle (18 hours photoperiod) for vegetative growth. After a desirable vegetative growth, plants were subjected to flowering. For the onset of flowering, plants were exposed to 12 hours photoperiod. From the beginning, plants were divided in two different groups, (1) control (n = 12) and (2) treated with 'vermicompost liquid extract' (n = 12). Fully matured plants of both groups (control and treated) were harvested (n = 9, from each group) for the analysis of biomass yield, cannabinoids and terpenes content. A schematic diagram of experimental design is shown in **Figure 1**.

Application of Vermicompost Liquid Extract

Plants of both groups (control and treated) were kept side by side under similar climatic controlled environmental conditions and, watered and fertilized normally. Well rooted vegetative cuttings grown in 4" biodegradable Jiffy pots were transplanted to normal size (~ 5 gallons) pots for further vegetative growth. Once transplanted to regular size pots, plants of group 2 (treated) were supplemented with 3 ounces of vermicompost liquid extract (Worm Power, Avon, NY) per plant per week till maturity.

Harvesting Drying and Processing

Fully mature plants were harvested, dried (overnight at 125 ± 5 ^{o}F) and processed individually for the estimation of usable dry biomass/plant. Biomass samples (*inflorescence* and leaves) of both the groups were analyzed and compared for cannabinoids and terpenes content.

Analysis of Cannabinoids and Terpenes Content

Biomass samples of twenty-four cannabis plants (12 control and 12 treated with vermicompost liquid extract) were used for cannabinoids and terpenes content. Seven cannabinoids {(Δ^8- tetrahydrocannabinol (Δ^8-THC), Δ^9- tetrahydrocannabinol (Δ^9 –THC), cannabidiol (CBD), tetrahydrocannabivarin (THCV), cannabinol (CBN), cannabigerol (CBG), and cannabichromene (CBC), **Figure 2**} and ten different terpenes (α-pinene, α-terpineol, β-pinene, β-Myrcene, β-caryophyllene, α-humulene, caryophyllene oxide, linalool, limonene and terpinolene, **Figure 3**) were quantitively analyzed using our previously published GC-FID methods (Eloshly et al 2016 and Ibrahim et al 2022).

Cannabinoids Analysis

GC-FID Instrumentation and Conditions for Cannabinoids Analysis

A gas chromatography (GC) analyses was performed using Varian CP-3380 gas chromatographs, equipped with Varian CP-8400 autosamplers, capillary injectors, dual flame ionization detectors, and DB-1MS columns (15 m × 0.25 mm × 0.25 μm) (J&W Scientific, Folsom, CA). Data was recorded using a Dell Optiplex GX1 computer and Varian Star workstation software (version 6.1). Helium was used as carrier and detector makeup gas with an upstream indicating moisture trap and a downstream indicating oxygen trap. Hydrogen and compressed air were used as the combustion gases. The following instrument parameters were employed: air, 30 psi (300 mL/min); hydrogen, 30 psi (30 mL/min); column head pressure, 15 psi (1.0 mL/min); split flow rate, 100 mL/min; split ratio, 50:1; septum purge flow rate: 5 mL/min; makeup gas pressure, 20 psi (30 mL/min); injector temperature, 240°C; detector temperature, 270°C; oven program, 170°C (hold 1 min) to 250°C at 10°C/min (hold 3 min); run time, 12 min; injection volume, 1 1L. The instruments are daily maintained and calibrated to ensure a Δ^9- THC/internal standard response factor ratio of one.

Calculation of Concentrations

The concentration of a specific cannabinoid is calculated as follows:

Cannabinoids (%) =
{GC area (cannabinoid) / GC area (ISTD)} × {Amount (ISTD) / Amount (sample)} × 100

Terpenes Analysis

GC-FID Instrumentation and Conditions for Terpenes Analysis

GC-FID analysis was performed on an Agilent 7890B GC system fitted with an autosampler 7693. Separation was performed using a DB5-MS (30 m x 0.25 mm I.D. 0.25 µm film thickness) (J&W Scientific Inc. Agilent technologies) column. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. and the FID make-up gas. The inlet was configured in split mode with a 15:1 split ratio and a temperature of 250˚C. The oven time program began at 70˚C for 2 min. before ramping at a rate of 3˚C/min. to 85˚C. The oven temperature was increased at a rate of 2˚C/min. to 165˚C and held for 1 min. before ramping at a rate of 30˚C/min. to 250˚C where it was held for 20 min. The total run time was approximately 60 min. The detector temperature was set at 300˚C and the hydrogen, air, and make-up flow rates were 40, 500, and 27 mL/min., respectively. Data analysis was performed using Agilent ChemStation® software (rev. B.04.02). The injection volume was 2 µL. All terpenes were recognized in samples by comparing their retention times with authentic references.

Standards and Reagents

All reference cannabinoids standards were purchased from Cayman Chemicals as 1 mg/mL solutions in MeOH with purity ≥95%. All terpenes standards were purchased from Sigma-Aldric with purity ≥ 95%. The purity of cannabinoids and terpenes was confirmed by GC/MS.

Results and Discussion

Composting is the purposeful biodegradation of organic matter, using micro-organisms and/or earthworms, producing good quality fertilizers rich in nutrients. The earthworms in the soil converts the organic wastes to rich compost called worm casting, vermicast or vermicompost, a nutritive soil amendment rich in microbial flora. It contains significant

quantities of nutrients, a large beneficial microbial population and biologically active metabolites, particularly gibberellins, cytokines, auxins and vitamins which can be applied alone or in combination with organic or inorganic fertilizers so as to get better yield and quality of crops.

We have evaluated the effect of the addition of a controlled dose of vermicompost with regular fertilizer on useable biomass yields, cannabinoids content and terpenes content of indoor grown *Cannabis sativa* L. plants. Vegetative cuttings of *Cannabis sativa* L. were made from high THC yielding mother plant. Cutting were kept under a climatic controlled condition for rooting and vegetative growth and flowering. Cuttings were provided 18 hours photoperiod during rooting and vegetative growth. After desirable growth plants were exposed to 12-hour photoperiod for initiation of flowering. From the beginning plants were separated in two groups control and treated. Both groups were watered and fertilized regularly and equally. However, treated plants were additionally supplied with 3 ounces of vermicompost liquid extract per plant per week, until maturity. At maturity, plants were harvested, dried, processed and evaluated for per plant biomass yield, cannabinoids and terpenes content in buds of both groups (control and treated) of plants.

Useable biomass yield in controlled and treated cannabis plants are shown in **Table 1**. A significant plant to plant variation in usable biomass was observed in both groups of plants ranging from 73.12 g. (clone ID 3) to 52.34 g. (clone ID 8) in the control plants and 78.14 g. (clone ID 3) to 68.19 g. (clone ID 7) in the treated plants. Average useable biomass yield per plant in control group was found to be 62.13 ± 7.32 whereas, it was recorded 71.50 ± 3.72 g. in the treated plants. In general, about 15 % increase in usable biomass yield per plant was obtained, when plants were treated with vermicompost. Similar results on effect of vermicompost on cereal, fruit and vegetable crops are reported by several authors (Beker et al., 1997; Beker et al., 2006; Palanisamy, 1996). In a glass house study, about 35% increase in *Triticum aestivum* (wheat) grain yield was reported by Beker et al. (1997) in worm-composted soil as compared to that of control. In another study, Beker et

al. (2006) reported an increase of about 47% (in wheat) and 51% (in soybean) in the crops yield by introducing earthworms in agricultural and reclaimed land situations. Palanisamy (1996) reported >40% increase in growth and yield of wheat crop by introducing vermicast to soil. A twofold increase in the grapes yield was reported by Buckerfield and Webster (1998) in soil treated with vermicompost. Similarly, higher yield of vegetable crops such as tomatoes (Atiyeh et al. 1999), okra (Gupta et al., 2008) and eggplants (Guerrero and Guerrero, 2006) was reported by the addition of vermicompost to the soil as compared to that of control plants.

Variation in different cannabinoids content in control and vermicompost treated plants of *Cannabis sativa* is shown in **Table 2**. Seven different cannabinoids namely Δ^8-THC, Δ^9 –THC, THCV, CBD, CBC and CBG and CBN were analyzed and compared among control and treated plants. Since this was a THC dominant variety, noticeable differences were evident in Δ^9-THC content as compared to other cannabinoids in both groups of plants. Among the control group, highest Δ^9-THC content (10.89%) was observed in clone ID 7 whereas the lowest (5.89%) was found in clone ID 5. In general, an increase in Δ^9-THC content was observed with highest of 12.42 % in clone ID 3 and lowest of 6.10% in clone ID 6 in the plants treated with vermicompost liquid extract. Average values of Δ^9-THC content was calculated to be 8.38 % in control plants whereas it was found to be 9.51 % in the group of plants treated with vermicompost. In general, average increase in Δ^9-THC content in treated plants was found to be 13.49 % higher than in the control plants.

Overall, based on the average increase in Δ^9-THC content per plant and average increase in plant biomass, about 30 % increase in the yield of THC per plant was observed when plants were treated with 3.00 ounce vermicompost liquid extract per plant per week till harvest. The higher yield of Δ^9-THC was therefore, attributed to the combined increase in the biomass on one hand and to the increase in the Δ^9-THC content per plant.

Variation in different terpenes content in the control and treated plants of *Cannabis sativa* is shown in **Table 3**. Ten different terpenes, α-pinene, α-terpineol, β-pinene, β-Myrcene, β-caryophyllene, α-humulene, caryophyllene oxide, linalool, limonene and terpinolene were analyzed using GC-FID method. There was no difference noted in the terpenes content of the control vs treated plants.

In conclusion, this study shows that plants treated with vermicompost liquid extract produced about 15% higher biomass as compared to that of control plants, and that there was an average increase in Δ^9-THC content per plant of 13.5 % for a combined increase in Δ^9-THC yield of ~30%. Therefore, based on the results it is evident that addition of vermicomposting can be beneficial for the growth and yield of *Cannabis sativa* plants.

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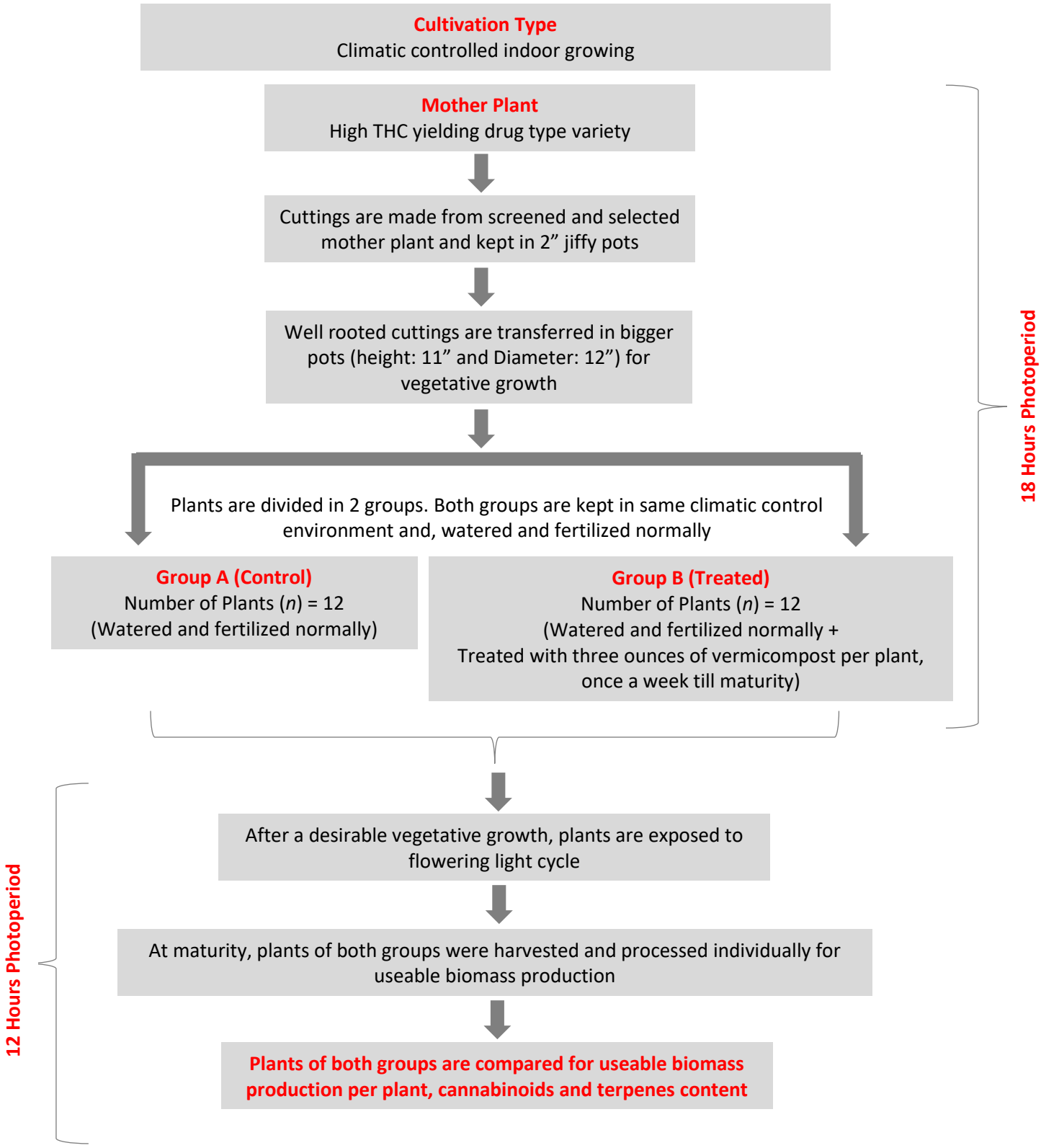

Figure 1: A schematic diagram of experimental design for the evaluation of the effect of vermicompost on useable biomass yield, cannabinoids and terpenes content of indoor grown *Cannabis sativa* L. plants

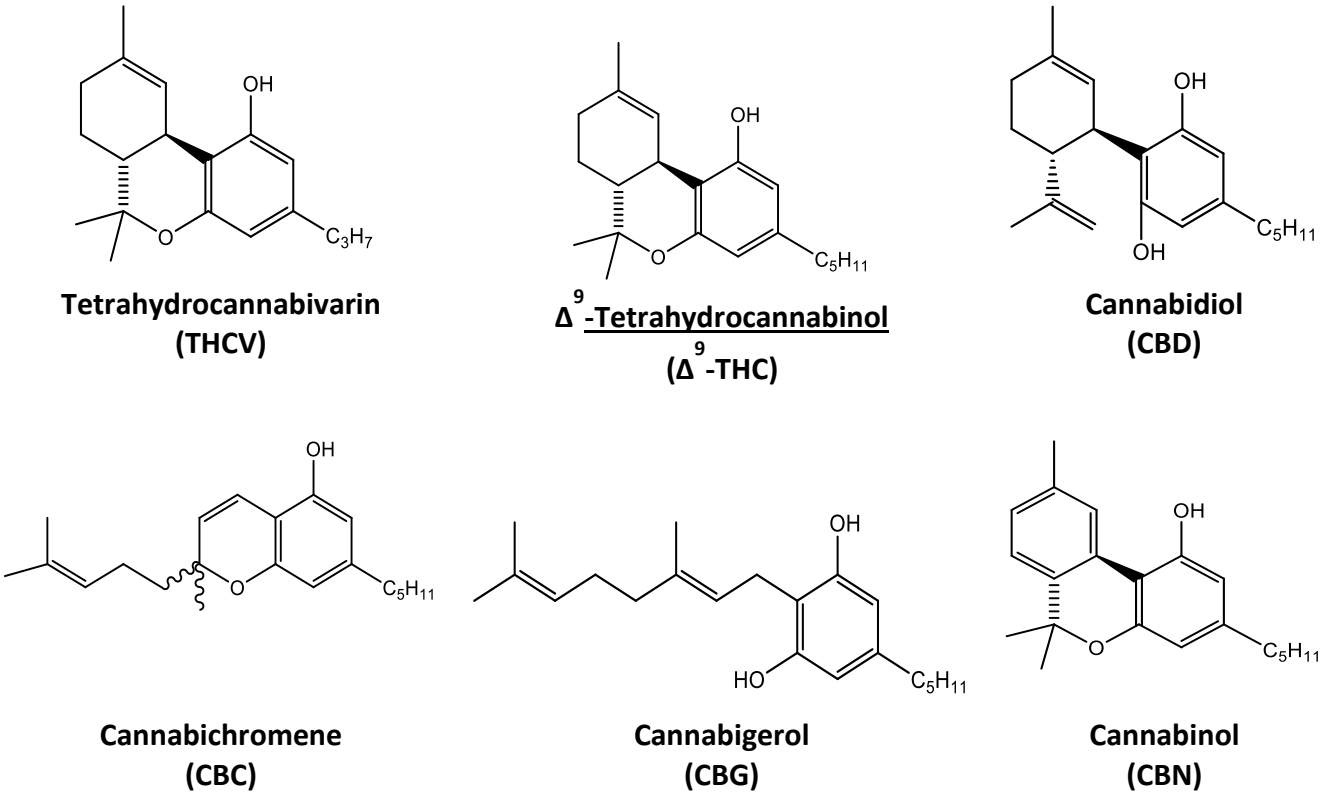

Figure 2: Chemical structures of major Phytocannabinoids in cannabis plant

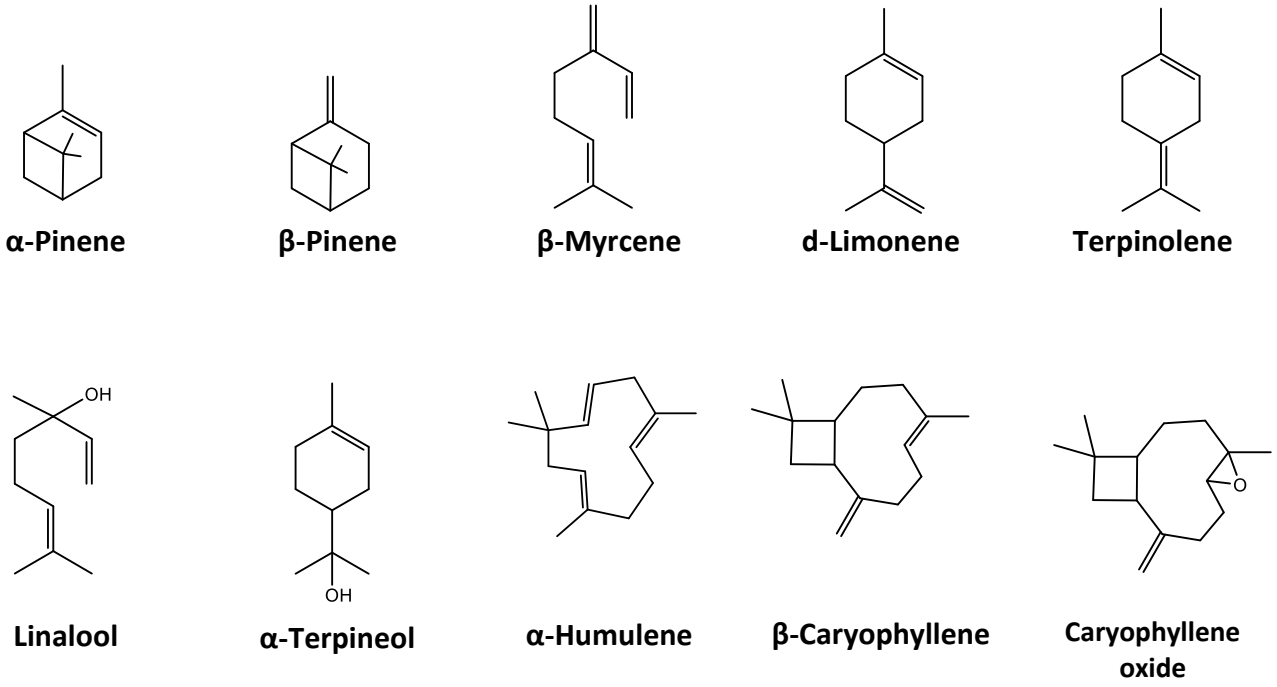

Figure 3: Chemical structures of major terpenes in cannabis plant

Table 1: Variations in useable biomass yield in control and vermicompost treated plants of *Cannabis sativa* L.

Clone ID	Weight of dried useable biomass/plant (g)
Control	
1	69.23
2	52.42
3	73.12
4	58.37
5	61.11
6	67.34
7	66.15
8	52.34
9	59.12
Mean ± SD	62.13 ± 7.32
Treated with vermicompost	
1	72.13
2	68.47
3	78.14
4	69.36
5	71.52
6	69.22
7	68.19
8	77.12
9	69.32
Mean ± SD	71.50 ± 3.72

Table 2: Variations in different cannabinoids content in control and vermicompost treated plants of *Cannabis sativa* L.

Clone ID	THCV (%)	CBD (%)	CBC (%)	Δ^{8} THC (%)	Δ^{9} THC (%)	CBG (%)	CBN (%)
			Control				
1	0.05	0.06	0.27	0.00	8.83	0.18	0.05
2	0.05	0.06	0.25	0.00	9.18	0.26	0.06
3	0.06	0.00	0.23	0.00	9.46	0.23	0.06
4	0.06	0.05	0.30	0.00	9.66	0.19	0.05
5	0.04	0.32	0.31	0.00	7.78	0.16	0.04
6	0.03	0.00	0.29	0.00	6.15	0.12	0.00
7	0.06	0.00	0.27	0.00	10.49	0.26	0.06
8	0.00	0.00	0.30	0.00	5.89	0.11	0.00
9	0.05	0.00	0.32	0.00	8.01	0.16	0.04
Mean ± SD	**0.04 ± 0.02**	**0.05 ± 0.10**	**0.28 ± 0.03**	**0.00 ± 0.00**	**8.38 ± 1.57**	**0.19 ± 0.06**	**0.04 ± 0.02**
			Treated with vermicompost				
1	0.07	0.00	0.22	0.00	11.06	0.33	0.10
2	0.05	0.00	0.17	0.00	7.31	0.24	0.09
3	0.08	0.05	0.27	0.00	12.42	0.39	0.09
4	0.07	0.05	0.25	0.00	10.84	0.22	0.07
5	0.04	0.00	0.28	0.00	6.40	0.09	0.05
6	0.04	0.00	0.25	0.00	6.10	0.11	0.05
7	0.06	0.00	0.24	0.00	9.62	0.24	0.07
8	0.07	0.00	0.24	0.00	10.61	0.23	0.07
9	0.07	0.05	0.22	0.00	11.22	0.27	0.07
Mean ± SD	**0.06 ± 0.01**	**0.02 ± 0.03**	**0.24 ± 0.03**	**0.00±0.00**	**9.51 ± 2.32**	**0.24 ± 0.09**	**0.00 ± 0.02**

Table 3: Variations in different terpenes content in control and vermicompost treated plants of *Cannabis sativa* L.

Clone ID	α-pinene (mg/g)	β-pinene (mg/g)	β-Myrcene (mg/g)	β-caryophyllene (mg/g)	α-humulene (mg/g)	Caryophyllene oxide (mg/g)
			Control			
1	0.74	0.58	0.01	4.63	0.00	0.41
2	1.30	0.97	0.01	6.68	0.00	0.66
3	0.84	0.59	0.01	4.00	0.00	0.40
4	1.05	0.77	0.01	5.23	0.00	0.43
5	0.86	0.64	0.01	4.28	0.00	0.42
6	0.97	0.71	0.01	5.46	0.00	0.54
7	1.02	0.72	0.01	4.97	0.00	0.43
8	1.60	1.12	0.01	8.21	0.00	0.64
9	1.17	0.80	0.01	5.94	0.00	0.46
Mean ± SD	**1.06 ± 0.26**	**0.77 ± 0.18**	**0.01 ± 0.00**	**5.49 ± 1.31**	**0.00 ± 0 .00**	**0.49 ± 0.10**
			Treated with vermicompost			
1	1.08	0.75	0.01	5.36	0.00	0.48
2	0.81	0.57	0.01	4.23	0.00	0.41
3	0.93	0.65	0.01	4.48	0.00	0.37
4	0.77	0.53	0.00	4.04	0.00	0.38
5	1.15	0.80	0.01	5.75	0.00	0.49
6	1.72	1.20	0.01	9.13	0.00	0.56
7	0.80	0.58	0.01	4.12	0.00	0.32
8	1.10	0.87	0.01	6.57	0.00	0.59
9	0.65	0.49	0.00	3.59	0.00	0.32
Mean ± SD	**1.00 ± 0.31**	**0.72 ± 0.22**	**0.01 ± 0.00**	**5.25 ± 1.74**	**0.00 ± 0.00**	**0.44 ± 0.10**

Limonene, Terpinolene, Linalool and α-Terpineol were found below the limit of detection.