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Variability of total THC in greenhouse cultivated dried Cannabis

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Natural variation in secondary metabolites is commonplace in organic matrices, including consumable fruits and vegetables. However, absolute concentration and variation of secondary metabolites are generally not of concern to consumers. *Cannabis sativa* and *C. indica* dried cannabis total Delta-9 Tetrahydrocannabinol (THC) is of importance to consumers/patients and must be accurately reported for both recreational and medical dried flower. In this report, the variation in Total THC in THC-dominant commercially relevant cultivars was investigated. The variation in Total THC values within different strata of the plant and between plants of the same batch were explored using a single analytical method. Within one stratum across nine batches ($n = 27–57$), Total THC varied by 3.1–6.7% of actual content, with only ~ 30–41% of individual replicates falling within their respective 99% confidence internal (CI) (representative of the batch mean). Between the top and bottom of plants across three batches, Total THC varied by 4.7–6.1% of actual THC content. Between plants of one cultivar, average Total THC varied by 2.8% which was statistically significant ($p < 0.0001$). Effect size (ES) measures were also reported for plant strata and plant against plant analysis. A comprehensive analysis of the extent of Total THC variation in samples of dried cannabis, evident both within and across plants of the same batch, has been presented using a random sampling and sample size calculated approach. Herein we demonstrate the natural variability present in dried cannabis flower using a single analytical method.

Keywords Secondary metabolites, Cannabinoids, Delta-9 Tetrahydrocannabinol, Potency, Total THC, Natural Variation, Cannabis

Variability in plant nutrient concentration is well documented in the literature. Beltramo et al. describe the concentration variation in minerals and vitamins of various fruits across different countries[1], and Sarker et al. describe the antioxidant vitamin and mineral variation in 25 genotypes of the vegetable Amaranth[2]. It is widely accepted that the variation in concentration of these biochemical compounds is expected. However, although consumed for their purported health benefits, fruits and vegetables are consumed without the requirement or interest in the absolute concentration of their vitamins and minerals.

The species *Cannabis sativa* and *Cannabis indica* of flowering plants in the family Cannabaceae are consumed for their psychoactive and non-psychoactive properties by both patients and recreational consumers. This paper is focused on dried cannabis flower, which is typically consumed via inhalation, i.e., smoking or vaping. In dried cannabis, the key psychoactive ingredient, (−)-*trans*-Δ^9-tetrahydrocannabinol (delta-9-THC), is present primarily in its acidic form, THCA, which is non-psychoactive[3]. THCA is converted to the psychoactive form, THC, via a decarboxylation reaction which is induced by the addition of heat, via combustion or vaporization[4]. Accurately reporting the total concentrations of the active ingredients in cannabis, THC and cannabidiol (CBD), is required in Canada as per the Regulations under the Cannabis Act. Total THC (calculated as [THCA] *0.877 + [delta-9-THC]) can represent up to one-third of the cannabis flower matrix (see Fig. 1, cultivar 'B17'). As the legal cannabis market continues to expand worldwide and shifts from solely medical use to include the recreational sector, quantifying Total THC with accuracy and precision is essential. The source of variability in Total THC found in dried cannabis is two-fold: at the level of the plant, and at the level of measurement in the laboratory. The variability in the latter has been well-documented: Coogan (2022) reported a variance of 20% in total THC in medical cannabis of different samples of the same production lot[5]. Smith (2019) discusses the common reasons behind inter-lab testing differences, including different sample preparation and analytical methods, sample handling, misuse of appropriate standards, and large dilution factors[6]. Label claims of legal

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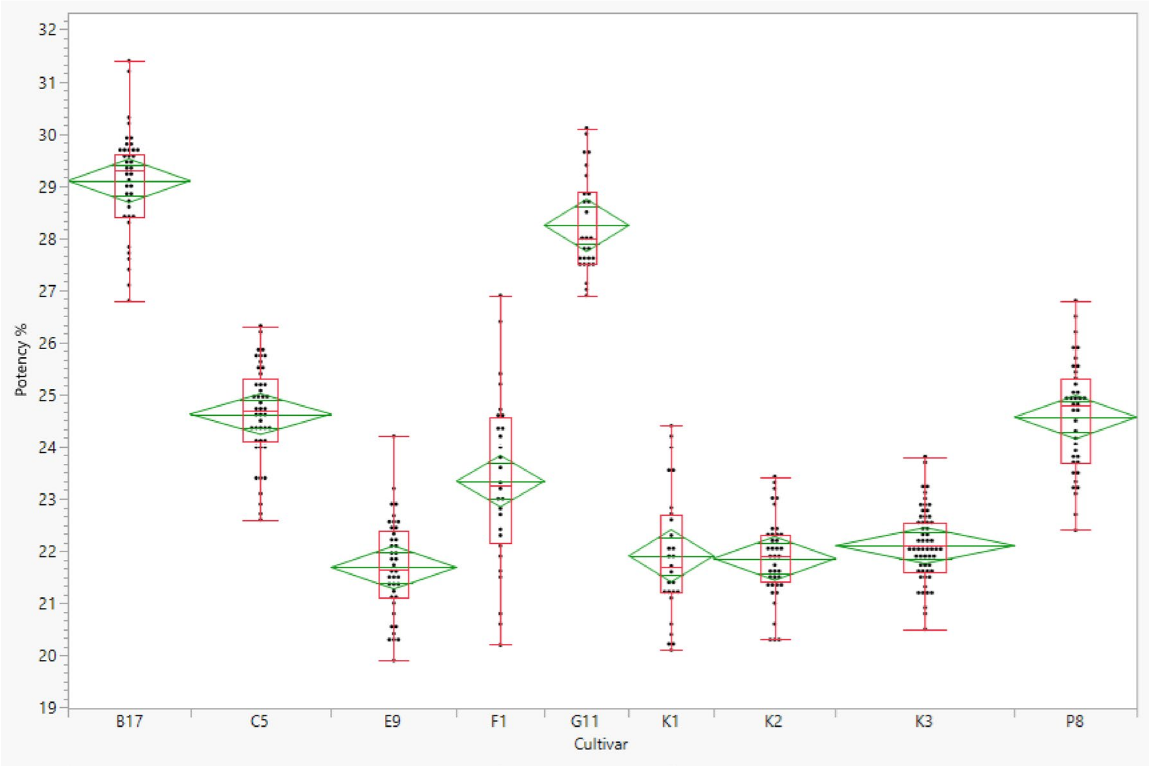

Fig. 1. Variation in Potency in a Single Stratum of Dried Cannabis Flower. Each black dot represents a single potency measurement (Total THC percentage). The range of each cultivar batch is delineated by the red box plots. Green diamonds are 99% CI. Values within these diamonds are good representations of the mean of the cultivar batch. Values outside these diamonds represent the cultivar batch, but not the mean.

cannabis product have been tested by independent laboratories in Canada. Doggett et al. (2024) investigated label accuracy of cannabis oil products and found 12 products (40%) were outside the listed variability limit for THC[7]. Results of an independent laboratory investigation into 46 legal dried cannabis flower products revealed that only three samples were within 15% of the label claim, whilst the other 43 were > 15% outside of the label claim, with deviations ranging up to ~ 50%[8]. By contrast, there is little in the literature describing total THC variability at the level of the plant, and to the best of our knowledge no investigations into total THC variability in dried cannabis flower have been performed and/or made publicly available.

Both CBD and THC are produced via the same biochemical pathway and stored in the secretory cavity of stalked glandular trichome heads[9]. Massuela et al. have described cannabinoid variation within different locations of a plant for total CBD[10] and Namdar et al. described how total THC content decreases as you move down the plant and is dependent upon the type of solvent used for secondary metabolite extraction[11]. CBD is of interest for its reported anti-inflammatory[12], anxiolytic[13], and analgesic[14] properties, for which THC is also used, however cannabis is traditionally consumed for the psychoactive effects of THC which has resulted in consumers paying close attention to the percentage of THC on the label, as it relates to their purchase decision. While THCA, the acidic form of THC, is not psychoactive, there is in vitro evidence to suggests its pharmacological activity with regards to potential anti-inflammatory[15], immunomodulatory[16], neuroprotective[17], and anti-neoplastic[18] properties.

In Canada, which became the first major industrialized country to provide legal and regulated access to cannabis for non-medical purposes on October 17, 2018, labelling for dried cannabis must include a single value, in mg/g, for each of THC and Total THC, that is deemed to be representative of the entire batch. However, it is not well understood how representative the value on the label is of either the potency of the entire batch, or of the dried cannabis in each commercial unit. By performing large random sampling of numerous THC-dominant cultivars coupled with potency testing using a single analytical method (for which the propagation of uncertainty is known – see Materials and Methods), three uncertainties were successfully addressed: (1) an understanding of the Total THC range from various cultivars from one stratum on the plant and how representative the values are of the sample mean, (2) an understanding of the Total THC range throughout the different strata of a plant, and (3) an understanding of the Total THC range throughout different plants of the same batch. To the best of our knowledge, we are the first to investigate the variability of Total THC across numerous THC-dominant cannabis cultivars using a large random sampling approach and its implications regarding single value Total THC reporting.

Cultivar ID	Mean	Min	25%	Median	75%	Max	Range %	99% CI	*N* replicates in 99% CI Diamond
B17	29.1%	26.8%	28.4%	29.3%	29.6%	31.4%	4.6%	28.7–29.5%	12/39 (30.8%)
F1	23.3%	20.2%	22.2%	23.3%	24.6%	26.9%	6.7%	22.5–24.2%	10/28 (35.7%)
P8	24.6%	22.4%	23.7%	24.8%	25.3%	26.8%	4.4%	24.1–25.0%	14/39 (35.9%)
C5	24.6%	22.6%	24.1%	24.7%	25.3%	26.3%	3.6%	24.3–25.0%	17/45 (37.8%)
E9	21.7%	19.9%	21.1%	21.7%	22.4%	24.2%	4.3%	21.3–22.1%	15/40 (37.5%)
G11	28.2%	26.9%	27.5%	28.0%	28.9%	30.1%	3.2%	27.7–28.7%	8/27 (29.6%)
K1	21.9%	20.1%	21.2%	21.7%	22.7%	24.4%	4.3%	21.3–22.6%	11/27 (40.7%)
K2	21.9%	20.3%	21.4%	21.9%	22.3%	23.4%	3.1%	21.5–22.2%	16/39 (41.0%)
K3	22.1%	20.5%	21.6%	22.1%	22.6%	23.8%	3.3%	21.9–22.4%	19/57 (33.3%)

Table 1. Single stratum sampling descriptive. Statistics. Box plot descriptive statistics and mean included for Fig. 1. 99% CI and the corresponding number of replicates included.

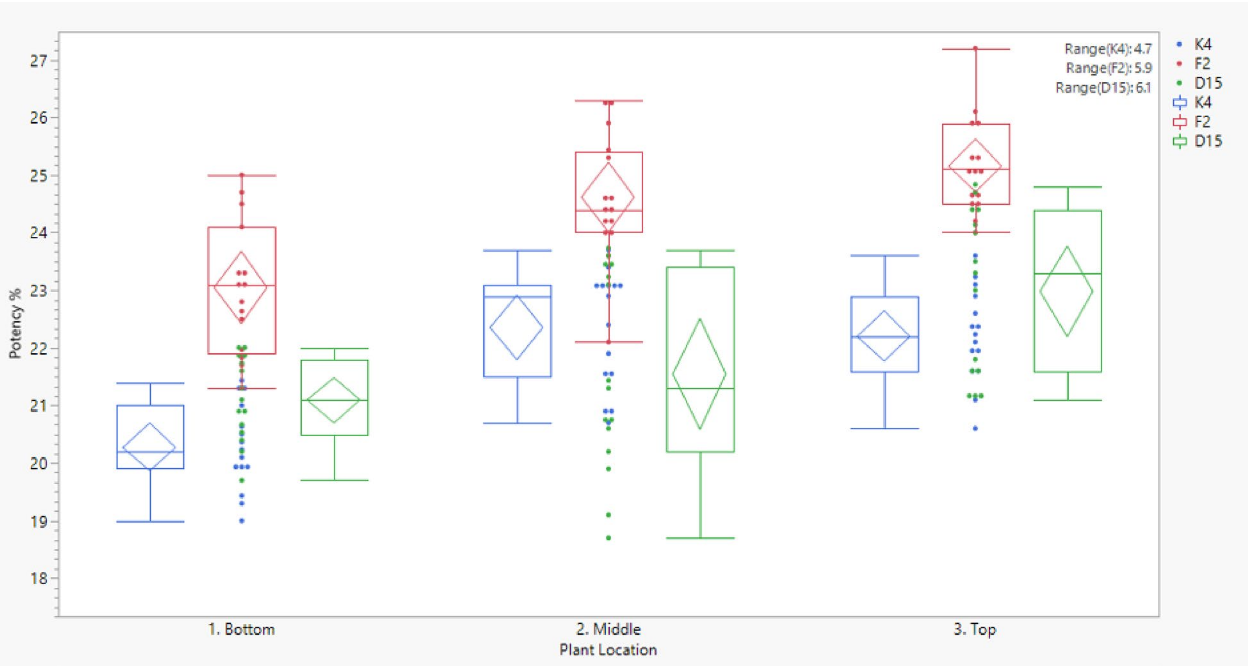

Fig. 2. Variation in Potency in Three Strata of Dried Cannabis Flower (Top, Middle, Bottom). Three different cultivars were analysed, and potency values were reported for the top, middle, and bottom strata of the dried cannabis plant. Each dot represents a single potency measurement (Total THC percentage). The range of each stratum is delineated by the box plots. The magnitude of the potency range for each cultivar is indicated in the top right corner of the graph.

Results

Potency variation from one stratum of plant

To assess potency variability within a single vertical layer of the plant, samples were taken from the tops of plants (see 'Sampling method' in Materials and Methods section) and each Total THC value measured was plotted as a scattergram in Fig. 1. See Table 1 for cultivar descriptive statistics for the data in Fig. 1; Table 5 in the Materials and Methods for sample sizes. Across nine batches (of seven different cultivars), between 29.6% and 41.0% of individual replicates fell within their respective 99% confidence diamonds (see Table 1 last column, see Fig. 1 green diamonds). These two extremes represented cultivars 'K2' and 'B17', which both have the same sample size ($n = 39$). The smallest potency spread observed was 3.1% ('K2') and the largest was 6.7% ('F1') (see Table 1).

Potency variation throughout top, middle, bottom strata of plant

To assess whether the height of a cannabis flower on the plant impacts potency, samples were taken from the top, middle, and bottom of plants (see 'Sampling method' in Materials and Methods section) and each Total THC value measured was plotted as box plots in Fig. 2 and descriptive statistics in Table 2. Total THC ranges for the entire plant for cultivars 'K4', 'F2', and 'D15' were 4.7%, 5.9%, and 6.1% respectively, which is comparable to the spread observed with the top sampling in Table 1 (3.1–6.7%). As shown with the box plots in Fig. 2, the top and middle strata potencies are higher than the bottom, but the Total THC spread is varied between each stratum

Cultivar ID	Plant location	Mean	Stratum 99% CI	*Reps in 99% CI	Plant 99% CI	**Reps in 99% CI	Min	25%	Median	75%	Max	Total THC % Range
K4	Top	22.2%	21.6–22.8%	13/45 (28.9%)	21.1–22.1%	12/45 (26.7%)	20.6%	21.6%	22.2%	22.9%	23.6%	3.0%
	Middle	22.4%	21.8–23.0%	12/45 (26.7%)			20.7%	21.5%	22.9%	23.1%	23.7%	3.0%
	Bottom	20.3%	19.7–20.9%	13/45 (28.9%)			19.0%	19.9%	20.2%	21.0%	21.4%	2.4%
F2	Top	25.2%	24.4–25.9%	22/45 (48.9%)	23.7–24.8%	19/45 (42.2%)	24.0%	24.5%	25.1%	25.9%	27.2%	3.2%
	Middle	24.6%	23.9–25.3%	25/45 (55.6%)			22.1%	24.0%	24.4%	25.4%	26.3%	4.2%
	Bottom	23.0%	22.3–23.8%	8/45 (17.8%)			21.3%	21.9%	23.1%	24.1%	25.0%	3.7%
D15	Top	23.0%	22.0-23.9%	10/45 (22.2%)	21.2–22.5%	15/45 (33.3%)	21.1%	21.6%	23.3%	24.4%	24.8%	3.7%
	Middle	21.5%	20.6–22.5%	22/45 (48.9%)			18.7%	20.2%	21.3%	23.4%	23.7%	5.0%
	Bottom	21.1%	20.1–22.0%	26/45 (57.8%)			19.7%	20.5%	21.1%	21.8%	22.0%	2.3%

Table 2. Top, Middle, bottom strata sampling descriptive statistics. Box plot descriptive statistics and mean included for Fig. 2. *The number of replicates from all plants that fell within one plant stratum average 99% CI. **The number of replicates from all plants in a cultivar batch that fell within the plant average 99% CI.

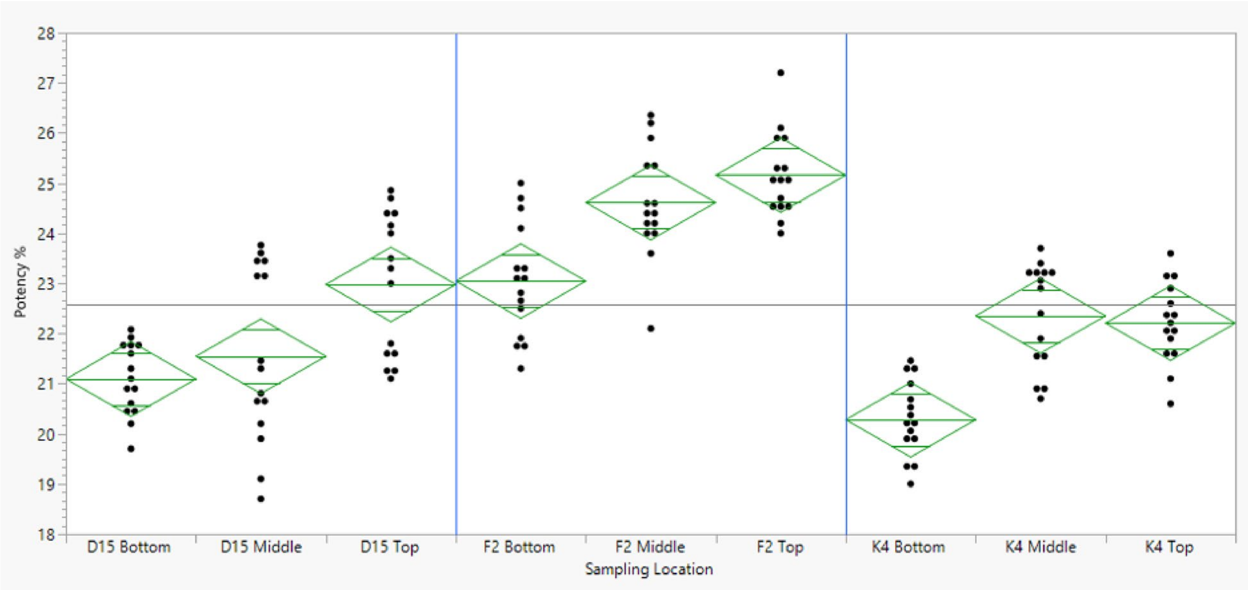

Fig. 3. Cultivars 'K4', 'F2', and 'D15' Top, Middle, Bottom Analysis. Blue lines separate each cultivar. One-way ANOVA ($p < 0.0001$) was performed, with Tukey-Kramer HSD post-hoc test for both cultivars 'K4' and 'F2'. For cultivar 'D15', Kruskal-Wallis ($p = 0.0012$) was performed, with Dunn's post-hoc test (see Supplementary Data for all post-hoc data). Each black dot represents a single potency measurement (Total THC percentage). Green diamonds represent 99% CI.

(see Table 2). Across all three cultivars, between 17.8 and 55.6% of replicates from throughout all stratums measured fell within one of the stratum's 99% CI, and between 26.7 and 42.2% of replicates across all plants for a cultivar fell within the plant average 99% CI (see Table 2).

The Total THC variation *between strata* of plants of the same cultivar from Table 2 was investigated and plotted in Fig. 3. For cultivar 'K4' and 'F2', a one-way ANOVA ($\alpha < 0.01$) with Tukey-Kramer HSD as the post-hoc analysis was performed due to equal variances (Bartlett $p > 0.01$). For cultivar 'D15', Kruskal-Wallis ($\alpha < 0.01$) with Dunn's as the post-hoc analysis was performed due to unequal variances (Bartlett $p = 0.0076$). To compare the magnitude of the statistical differences observed, effect size (ES) measures were calculated and reported using Cohen's d for all measures except 'cultivar 'D15' middle versus bottom' which used Glass's Delta due to unequal variances (confirmed by Fischer's F test, see Tables 1 and 2 in Supplementary Data) as reported in Table 3, alongside strata mean Total THC in brackets.

Regarding statistical significance, for both cultivars 'K4' and 'F2', top and middle strata are statistically different from the bottom stratum with regards to average potency % ($p < 0.0001$ and $p = 0.0004$, respectively). For cultivar 'D15', top stratum is statistically different than the bottom stratum with regards to average potency % ($p = 0.0058$). While p-values can inform whether there is an effect, it does not describe the size of the effect. Statistical significance can simply be achieved using large sample sizes, hence the inclusion of effect size which is independent of sample size.

K4	Top	Middle	Bottom	F2	Top	Middle	Bottom	D15	Top	Middle	Bottom
Top	N/A	0.22 (22.2 vs. 22.4%)	2.45 (22.2 vs. 20.3%)	Top	N/A	0.61 (25.2 vs. 24.6%)	2.20 (25.2 vs. 23.0%)	Top	N/A	0.96 (23.0 vs. 21.5%)	1.71 (23.0 vs. 21.1%)
Middle	N/A	N/A	2.36 (22.4 vs. 20.3%)	Middle	N/A	N/A	1.45 (24.6 vs. 23.0%)	Middle	N/A	N/A	0.23* (21.5 vs. 21.1%)
Bottom	N/A	N/A	N/A	Bottom	N/A	N/A	N/A	Bottom	N/A	N/A	N/A

Table 3. Cultivars 'K4', 'F2', and 'D15' effect sizes. Cohen's d effect size measures, where > 0.8 is considered large and > 1.2 is considered very large. Strata mean total THC percentage included in brackets. *For middle versus bottom for 'D15', Glass's Delta was used.

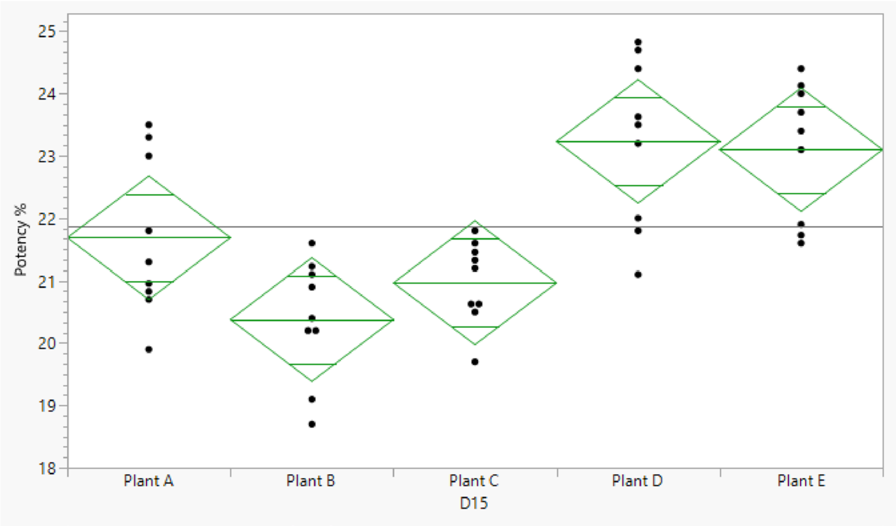

Fig. 4. Left: Cultivar 'D15' Plant ANOVA and post-hoc analysis. One-way ANOVA ($p < 0.0001$) was performed, with Tukey-Kramer HSD post-hoc test (see Supplementary Data for post-hoc data). Plants D and E are both statistically different from plants B and C ($p < 0.001$ across all comparisons).

Regarding effect size measures, for cultivar 'K4', the top and middle average Total THC value is greater than the bottom (ES 2.45 and 2.36, respectively). For cultivar 'F2', the top and middle average Total THC value is also greater than the bottom (ES 2.20 and 1.45, respectively). The top and middle strata were statistically comparable for both cultivars 'K4' and 'F2'. For cultivar 'D15', only the top and bottom were statistically different (ES 1.71). For the top and middle strata, although they are marginally statistically equivalent, the ES of 0.96 is large, so this can still be considered a large difference. Unlike cultivars 'K4' and 'F2' data, the average Total THC for the middle and bottom strata were very similar at 21.5% and 21.1%, respectively.

Potency variability amongst different plants from the same cultivar

To understand the variability *between plants* of the same cultivar, an analysis was conducted for a single cultivar. Using the data for cultivar 'D15', five plants ($n = 9$) used in the analysis were plotted against each other and compared by one-way ANOVA ($p < 0.0001$) (see Fig. 4), with Tukey-Kramer HSD as the post-hoc analysis due to equal variances (Bartlett $p = 0.3837$). Plants D and E are statistically different to both plants B and C even though they came from the same cultivar ($p < 0.001$ across all comparisons).

The ES matrix in Table 4 compares cultivar 'D15' plants against each other. Fischer's F-test confirmed the variances were equal, so Cohen's d was used as the ES measure, with values > 0.8 showing a large difference between groups. There was a large difference in Total THC observed between plants D and E with both B and C (ESs of 2.41 and 2.11, and 2.57 and 2.28, respectively). Plant A was not statistically different to plants B, D, and E but displayed large ESs (1.12, 1.15, and 1.16, respectively). The 99% CI for each plant in cultivar D15, and how representative it is of the other measured plants, was captured and reported in Table 4. Between 33.3 and 88.9% of replicates from a single plant fell within the respective plant's 99% CI, whereas between 33.3 and 72.2% of replicates from the other four plants fell within a single 99% CI.

Discussion

The work presented here captures natural variation of Total THC (potency) in dried cannabis. The analysis was three-fold: (1) single strata sampling using samples from the top of the plant to investigate how representative single value potency percentage reporting is of an entire cultivar batch, (2) top, middle, and bottom bud sampling to investigate the extent of potency variation across a single dried cannabis plant (and how representative a stratum was of the other strata), and (3) plant versus plant sampling from the same batch to investigate the extent

ES matrix	Plant A	Plant B	Plant C	Plant D	Plant E	Plant vs. Plant	99% CI	*Reps in 99% CI	**Reps in 99% CI
Plant A	N/A	1.12	0.67	1.15	1.16	A	20.7–22.7	5/9 (55.6%)	15/36 (41.7%)
Plant B	N/A	N/A	0.70	2.41	2.57	B	19.4–21.4	6/9 (66.7%)	18/36 (50.0%)
Plant C	N/A	N/A	N/A	2.11	2.28	C	20.0–22.0	8/9 (88.9%)	26/36 (72.2%)
Plant D	N/A	N/A	N/A	N/A	0.08	D	22.2–24.2	3/9 (33.3%)	12/36 (33.3%)
Plant E	N/A	N/A	N/A	N/A	N/A	E	22.1–24.1	5/9 (55.6%)	11/36 (30.6%)

Table 4. Effect size (ES) Matrix: cohen's d effect size measures where > 0.8 is considered large and > 1.2 is considered very large. Plant versus plant. How representative a single plant is of other plants from cultivar 'D15'. *The number of replicates from the same plant that fell within its own 99% CI. **The number of replicates from other plants that fell within a single plant's 99% CI.

of the potency variation across plants (and how representative a plant was of other plants in the same cultivar batch). At all levels of analysis, the variation in potency ranged from a minimum of 2.4% to a maximum of 6.7% of THC.

For the single stratum analysis, 30–41% of individual replicates were within the 99% CI diamonds. As the sampling performed was random and sample sizes were ≥ 30, these 99% CI ranges can be considered good estimations of the population mean due to the central limit theorem. If 30–41% of Total THC values are within this limit, the rest cannot be considered a good representation of the mean as stand-alone values. This could be seen as problematic, as even though these are observed potency values and are representative of what is possible from a cultivar batch, depending upon sampling and the extent of variation within an individual cultivar batch, repeated testing of different samples using different methods could be expected to yield different results.

For the multiple strata analysis, plants were stratified into top, middle, and bottom. The top and middle strata were consistently higher in potency than the bottom stratum. Total THC for cultivars 'K4, 'F2', and 'D15' ranged by 4.7%, 5.9%, and 6.1%, respectively. Top, middle, and bottom Total THC data demonstrated that the Total THC amongst some strata were statistically different while others were comparable. The statistically different strata in the same plant could be considered different potency populations. This could be due to cannabis buds lower down the plant receiving less light due to heavy shielding from dense foliage, while the top and middle cannabis buds have better exposure to UV irradiation[19]. Further, it has been observed that different pruning techniques affect the chemical uniformity of the plant[20,21]. Between 17.8 and 55.6% of replicates throughout all strata measured fell within one of the stratum's 99% CI. Between 26.7 and 42.2% of replicates across all plants of a cultivar batch fell within the plant's average 99% CI. This raises a similar issue as discussed above; if 50% or less of the replicates cannot be accounted for by the 99% CI, then these are not representative of the mean. Interestingly, across the three cultivars, different strata were more representative of all measured potencies: Cultivar 'K4' top and bottom (both 28.9%), cultivar 'F2' middle (55.6%), and cultivar 'D15' bottom (57.8%). To determine if this analysis was adequate at detecting a difference between groups, adjusted retrospective power was calculated for cultivars 'K4', 'F2', and 'D15' yielding 0.99, 0.99, and 0.71, respectively. (see Supplementary Data for raw data). It is important to note that typically samples from top, middle and bottom would be mixed without consideration of this variation.

For the final analysis, different plants ($n = 5$) from cultivar 'D15' were compared against each other using pooled data from the top, middle, and bottom. A one-way ANOVA test demonstrated that the plants were statistically different, with Tukey-Kramer HSD post-hoc test suggesting plants D and E were different from plants B and C. The average potency ± 1 SD of these plants was 23.2 ± 1.3% and 23.1 ± 1.1%, and 20.4 ± 1.0% and 21.0 ± 0.7%, respectively, with a difference of 2.8% between the potency averages of the highest and lowest plants. Adjusted retrospective power was also calculated for this analysis, yielding 0.99 (see Supplementary Data for raw data). 33–66% of Total THC replicates across four plants could be accounted for by a single plant 99% CI in this dataset.

For both the top, middle, and bottom strata and plant against plant analysis, the effect sizes for the statistically significant observations were very large, however there was discordance between some statistically insignificant results and effect sizes. This demonstrates the importance of not solely relying on statistical significance as a measure of difference; effect sizes are more robust at demonstrating where there is an actual meaningful difference.

The Total THC variation is present across plants, within plants, and within a single stratum of plants. For example, the pooled mean difference between the top and bottom of cultivar 'K4' plants was 2.1% Total THC, and the mean difference across the plants was 1.4%.

Adjusted retrospective power analysis was used for the top, middle, and bottom and plant versus plant analysis, as no prospective sample size calculations were performed. Determining statistical power bolsters the reliability of a dataset. The authors exercise caution when interpreting retrospective power analysis and are aware of its limitations, hence adjusted retrospective power analysis was used. Adjusted retrospective power analysis makes partial corrections for the positive bias introduced into retrospective power analysis due to the use of sample estimates[22]. This is achieved using a probability-weighted estimate that accounts for effect size uncertainty by considering the confidence interval range and averages the power over the range of uncertainty. It considers that the difference between samples can be within the whole CI range. Such an adjustment is appropriate for small samples sizes and offers a more conservative estimate of true effect size and thus study power.

Considering the random sample sized approach (analysis 1), retrospective power analysis of 0.71–0.99 (analysis 2 & 3), and the propagation of uncertainty for Total THC measurement being ± 0.2% (see Materials & Methods for calculation), confidence can be placed in the results presented as true natural variation. Furthermore, least significant number analysis demonstrates that between 17 and 30 replicates are required to observe a significant difference between groups and therefore the extent of variation in a particular cultivar batch (see Supplementary Data for raw data). To the best of our knowledge this paper represents the first comprehensive analysis into (a) the representativeness of single value Total THC reporting as compared to larger sample sizes, (b) the extent of Total THC variation throughout and across cannabis plants in multiple commercially-relevant THC dominant cultivars, and (c) the representativeness of one plant stratum versus other plant strata and one plant versus other plants.

The data presented provides valuable insight into the nature of Total THC variation within and across cannabis plants of the same cultivar batch. It is important to be aware of this natural variance when classifying and labelling dried cannabis for both recreational and medical use. A better understanding of potency variability in the plant presents an important opportunity to better inform cannabis patients, consumers, medical professionals, and regulatory bodies. The cannabinoid and terpene synthesis pathways[23], secondary metabolite storage[24], and the mechanism of action by which cannabinoids act at their receptor have been well documented in the literature[25]. However, the cannabis consumption experience is very subjective and complex, often being attributed to the 'entourage effect' first described in 1998 by Ben-Shabat and colleagues[26]. It contemplates major cannabinoids (i.e., THC, CBD), terpenes and minor and rare cannabinoids, as well as other compounds in the cannabis plant such as flavonoids, acting synergistically to produce the intoxicating and euphoric effects reported by consumers[27]. These effects exist on a continuum and can vary drastically between people, much like how the capsaicin content of chilli peppers and the reported Scoville heat rating intensity and experience post-consumption can vary[28]. The subjective experience of dried cannabis consumption is well known and reported[29–31], however objective cannabinoid testing outcomes (Total THC reporting) using large sample sizes and multiple cultivars has not been documented in the scientific literature. A single value reported on the label is only one of many possible values and is only representative of the mean occasionally. Although statistical analysis and effect size measures can quantify differences between groups, they do not give insight into what is deemed a meaningful difference in Total THC for consumers or regulatory bodies. However, from this dataset it can be concluded that there is no absolute value for Total THC that represents a batch and that reported Total THC can vary substantially within and across plants.

Materials and methods

Cultivation method. All cultivars selected for this study were THC-dominant, and contain little to no measurable CBD, CBDA or other minor and rare cannabinoids. Cannabis plants were grown under hydroponic cultivation as described elsewhere[32] for approximately 49 ± 1 days. Branches bearing flowers were hand-harvested, and hang-dried for six days as described[33]. All samples across all cultivars were taken at the same time point at the end of the hang dry process. To control for possible confounding variables, all batches of cultivars sampled were subject to the same temperature and humidity conditions in dry rooms of the same dimensions and plant fill density. Dry room conditions such as temperature and humidity were tightly controlled by the PRIVA greenhouse monitoring system and limits for excursions were the same across all sampled dry rooms.

Sampling method. 60–100 g of sample were taken randomly from 12 batches of eight different cultivars from hang-dry rooms (see Fig. 5 for plant stratification method and hang-dry sampling strategy). Cultivars were selected for this study based on their commercial relevance and to ensure that the observations recorded were applicable across multiple THC-dominant cannabis genetics, representative of what consumers could expect to find on the market. Samples were taken randomly from the ends of branches within each stratum. Three of these 12 batches had samples taken from the top, middle, and bottom of plants to investigate Total THC at different locations on plants and to compare plant to plant variability, and the remaining nine batches only had samples taken from the top stratum. See Table 5 below for a breakdown of the cultivar IDs and sampling. 'Total THC %' and 'potency %' are used interchangeably throughout this manuscript. As the vast majority of THC in dried cannabis is present in the acidic form (THCA), with very little measurable THC present in its neutral form (delta-9-THC), it was not pragmatic to quantify delta-9-THC and THCA variability separately.

All experimental research on plants was performed in accordance with the relevant guidelines, regulations and legislation. Pure Sunfarms Corp. is a licensed cultivator, processor and seller of cannabis under the Canadian Cannabis Act. This includes the right to perform research and development on cannabis.

Reagents. HPLC-grade water, acetonitrile, ammonium formate, formic acid, and extraction solvents and diluents were purchased from Fisher Scientific (Ottawa, ON, Canada).

Calibration Standards. Certified reference materials were manufactured by Cerilliant Corp. (Round Rock, TX) and purchased through MilliporeSigma Canada Ltd. (Oakville, ON). Each of the ten cannabinoids were provided individually as a 1.000 mg/mL solution.

Method Validation. Prior to data collection, the HPLC and sample preparation method was validated to ICH Q2(R2) Guidelines (2022)[34] and included Δ^9THC and THCA-A. Resolution for all analytes was > 1.7 as reported by Agilent OpenLab Data Analysis (USP). Calibration curve ranges for each analyte were prepared to accurately assess the in-sample analyte levels with appropriate weighting model applied based on residual plots. The limit of quantitation for all analytes was 0.1% w/w.

HPLC Analysis. An Agilent 1200 series HPLC system equipped with a temperature-controlled autosampler, binary pump, and diode array detector was utilized for cannabinoid analysis. Separation was achieved on a 150 × 3.0 mm 2.7 µm C18 column. Mobile phase composition was (A) 5 mM ammonium formate 0.1% formic acid in H_2O, and mobile phase (B) 0.1% formic acid in acetonitrile. Column temperature was set at 30 °C, flow rate at 0.7 mL/min as follows: 72.5% B 0.00–4.00 min, 85% B 4.01–6.00 min, 72.5% B 6.01–8.50 min. Detection was performed at 232 nm and 260 nm. The autosampler was maintained at 4 °C.

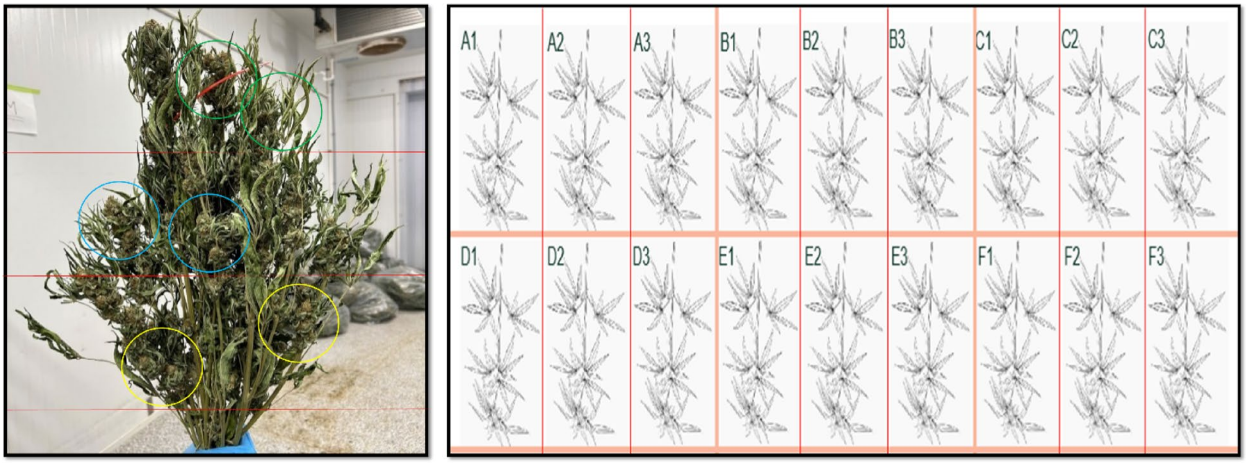

Fig. 5. Left: Plant Top, Middle, and Bottom Stratification. Plants were roughly split into three strata, and one bud was taken from the top (green), middle (blue), and/or bottom (yellow) stratum depending upon the analysis. Right: Hang Dry Plant Sampling Strategy. Lines represent netting to which plants were attached. Samples were split into blocks of three from beginning to end and top to bottom of netting with one location randomly picked using a random number generator from each block until a total of 60–100 g were collected. Only one plant per block was picked, to avoid randomly collecting samples from the same area of the room. For top bud only analysis, only top buds were collected (1 per plant), for top, middle, bottom analysis only one bud was collected per stratum per plant.

Cultivar ID	Total sample size (no. of 1 g replicates measured for total THC %)	Sample location
B17	39	Top only
F1	28	
P8	39	
C5	45	
E9	40	
G11	27	
K1	27	
K2	39	
K3	57	
K4	45 ($n = 15$ per location)	Top, Middle, Bottom
F2	45 ($n = 15$ per location)	
D15	45 ($n = 15$ per location)	

Table 5. Cultivar identification (ID) and sampling. All cultivar ids, sample sizes, and sample location.

Sample preparation. After buds were removed from plants, they were manicured to remove sugar leaves and stems. Approximately 1 g of sample was weighed into a 50 mL centrifuge tube for each replicate using a semi-micro- balance (Mettler Toledo) with draft shield. Samples were given appropriate identification numbers by one analyst and measured randomly using block randomisation by another analyst. 25 mL of two-part organic solvent (5:95) was dispensed into 50 mL centrifuge tubes using a grade A volumetric pipette and samples were agitated. Approximately 3 mL of supernatant was filtered using a syringe with 0.2 µm PTFE filter and diluted accordingly one time using 80% methanol. Samples were vortexed for approximately 8s and transferred to the HPLC sampler. To maintain consistency, analyst BC weighed out all samples & analyst KM dispensed all solvent and pipetted all analyte.

Statistical analysis

First Analysis. Sample size for top bud sampling was calculated using G*Power v. 3.1.9.7 using the input '*Difference from constant: One sample case*' (two-tailed, α 0.01, β 0.90, Cohen's d 0.8). For these inputs, a minimum sample size of 27 replicates per group was required. An alpha level (α) of 0.01 was chosen as this is more stringent than the classically used 0.05. A Cohen's d of 0.8 was used for sample size calculation because a large difference was expected; this was based on the CBD data presented in Massuela et al.[10] and pilot data collected in-house (data not shown).

Second & Third Analysis. One-way ANOVA was conducted for both the second and third analyses. Equality of variance checked using Bartlett's test, and Tukey-Kramer HSD as the post-hoc test. Kruskal-Wallis with

Dunn's as the post-hoc analysis was performed where variances were unequal. An α of 0.01 was used for all the above tests. Sample size calculations were not performed for the second & third analyses, therefore adjusted retrospective power analysis was conducted using the 'adjusted power' tool in JMP software. Justification for using adjusted retrospective power analysis is captured in the Discussion. See Supplementary Data for all test assumptions, including normality, outlier checking, and homogeneity of variances ANOVA analysis.

Propagation of uncertainty in the analytical method was calculated at ± 0.2% Total THC using equations obtained from the textbook Quantitative Chemical Analysis, 7th edition, Chap. 3[35]. Statistical analysis and graphing were performed using JMP v. 18.0.1.

Data availability

The datasets used for this formal analysis are available from the corresponding author upon request.

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Declarations

Competing interests
The work has been entirely funded by Pure Sunfarms Corporation and all authors are full-time employees of Pure Sunfarms Corp. The authors declare no additional competing interests, financial or non-financial.

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