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Organic nutrients and their potential to enhance agronomic performance measures and cannabinoid levels in cultivated *Cannabis sativa* L.

Kiriya Sungthongwisesa[1*], Tiwaporn Sudsanga[1], Chanon Lapjit[2,3], Jakkapat Paluka[4], and Khomsorn Lomthaisong[5]

[1]Agronomy section, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand
[2]Horticulture section, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand
[3]Cannabis Research Institute, Khon Kaen University, Khon Kaen, Thailand
[4]Cannabis Research Institute, Khon Kaen University, Thailand
[5]Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand

[*]Corresponding author: Kiriya Sungthongwisesa

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Abstract Farmers in Thailand are improving agricultural practices and organic approaches in crop production. When cultivating cannabis for cannabinoids, organic nutrients are thought to be better than inorganic options. This study investigates the effect of organic and inorganic nutrients on the growth and cannabinoid yield of *Cannabis sativa* KKU05 under field conditions. We tested four fertilizer treatments: no fertilizer, chicken manure, vermicompost, and inorganic nitrogen-phosphorous-potassium (NPK) fertilizer. Unfertilised plants yielded the lowest concentrations of cannabidiol, while plants fertilised with inorganic NPK or vermicompost performed the best, with cannabidiol concentrations of 4.94% and 4.91%, respectively. Vermicompost application was also associated with significantly higher concentrations of Δ9-tetrahydrocannabinol (0.21%) and cannabidiol (4.91%) compared to the no fertilizer method. There were no statistically significant differences in leaf and floret biomass or omega fatty acid concentrations among the chicken manure, vermicompost, and inorganic NPK fertilizer methods. This study demonstrates that vermicompost has excellent potential to improve cannabis yields for the production of cannabinoids under Good Agricultural Practices.

Keywords: Cannabinoid, Chicken manure, Fertilizer, Industrial hemp, Vermicompost.
Abbreviations: CBD_cannabidiol; CBN_cannabinol; DAT_days after transplanting; DTPA_diethylenetriaminepentaacetic acid; FID_flame ionization detector; GAP_good agricultural practices; GC_gas chromatography; H_2SO_4_2,2-dimethoxy propane; HPLC_high-performance liquid chromatography; ICP-OES_inductively coupled plasma optical emission spectroscopy; KKU_ Khon Kaen University; LEDs_light emitting diodes; NH^+_4_ammonium; NO^-_3_nitrate; PDA_Photodiode array; PTFE_Polytetrafluoroethylene; SPAD_soil plant analysis development; TFA_trifluoroacetic acid; THC_Δ9-tetrahydrocannabinol.

Introduction

Cannabis sativa L., or hemp, was grown for its natural fiber for centuries before cultivation was legalized in Thailand in 2020 (Seemakram et al., 2022). Due to its recent legalisation, *C. sativa* holds potential for industrialized cultivation (Pietrini et al., 2019) for pharmaceuticals and cosmetics (Grassa et al., 2018), bioenergy, animal feeds, and improvements to soil, including phytoremediation of heavy metals and other contaminants (Placido and Lee, 2022; Golia et al., 2023). *Cannabis sativa* typically contains very little THC, usually less than 0.3% (Velechovský et al., 2021) and more than 2% CBD. *Cannabis sativa* KKU05 is the first cannabis hybrid certified by Khon Kaen University in northeast Thailand. The KKU05 hybrid is rich in phytocannabinoids and performs well in high carbon and heavy metal growing conditions. Notorious phytocannabinoids such as cannabidiol (CBD), cannabinol (CBN), and Δ*9*-tetrahydrocannabinol (THC), are largely found in the glandular trichomes of female cannabis inflorescences (Barrales-Cureño et al., 2020). The seeds are useful to food industries and human health as they contain 25-35% oil (Farinon et al., 2020) and are high in essential fatty acids (Alonso-Esteban et al., 2023). In fact, this seed is the best source of omega 3, 6, and 9 (Seemakram et al., 2022). Omega-3 benefits are plentiful: research shows it can promote cardiac and ocular health, reduce inflammation, boost mental well-being, reduce the risk of Alzheimer's, support weight management, create a healthy complexion, and improve bone and joint health (Thomas et al., 2015). Omega-6 can improve brain function and cardiac health, decrease blood pressure, stimulate skin and hair growth, and contribute to orthopaedic health, while omega-9 can decrease inflammation and reduce the risk of heart disease and stroke (Thomas et al., 2015). Endocannabinoids are associated with airway relaxation and the reduction of atherosclerosis and hypertension, particularly in lung cancer patients (Tiffany et al., 2023). As the products of cannabis are increasingly used for medicinal purposes, Good Agricultural Practices (GAP) and organic standards are increasing the quality of cannabis produced (Ilikj et al., 2020).

Fertilizer management is key to the performance of cannabis plants under current safety standards. Nitrogen is a major abiotic variable affecting the developmental, physiological, and metabolic processes (Saloner and Bernstein, 2020; 2021) of cannabis seed, fiber, and biomass more than phosphorus and potassium fertilizers under field conditions (Deng et al., 2019; Wylie et al., 2021). Wylie et al. (2020) suggest that nitrogen fertilizer application should range between 60-200 kg ha^{-1}, phosphorous between 30-120 kg ha^{-1}, and potassium 40-200 kg ha^{-1} for industrial cannabis production, depending on expected yield and accounting for soil type and fertility status. During the reproductive growth phase, low inputs of nitrogen and phosphorous promote cannabinoid and terpenoid production, and increasing nitrogen and phosphorous application generally decreases the plants' secondary metabolism (Shiponi and Bernstein, 2021). In addition, concentrations of most cannabinoids and terpenoids decline when potassium application increases (Saloner and Bernstein, 2022). However, the form of nitrogen available from ammonium (NH^+_4) versus nitrate fertilizer (NO^-_3) is a major factor in cannabis physiological function and the biosynthesis of cannabinoids and terpenoids. Saloner and Bernstein (2022) demonstrated a dramatic 46% increased inflorescence yield when NH_4 was increased from 0 to 50%. However, moderate levels (10-30%) of NH_4 are suitable for medical cannabis cultivation, with little adverse influence on inflorescence and cannabinoid production. Consequently, 30% NH_4 is not recommended, as it increases the potential for severe or fatal NH_4 toxicity, damaging inflorescence yield, and cannabinoid production (Saloner and Bernstein, 2022). Nitrate is generally higher than NH^+_4 in vermicompost compared to chicken manure (Huang et al., 2016). Vermicompost and cow and chicken manure are biological, organic, environmentally friendly sources of nutrients and renewable energy, as well as low-cost inputs for crop production. Vermicompost and cow manure were tested for cannabis production and lack some of the benefits demonstrated for chicken manure, because dry-matter chicken manure contains comparatively more phosphorous, potassium, calcium, and magnesium (Anandyawati et al., 2023).

Some reports still advocate for large quantities of inorganic NPK fertilizer in cannabis cultivation. To achieve long-term sustainable yield, cannabinoid production, and soil fertility in industrial cannabis production, the performance of organic fertilizers compared to inorganic fertilizers must be investigated. Vermicompost and chicken manure are excellent sources of organic matter with high concentrations of macronutrients. Vermicompost improves nutrient content and other soil properties (Anandyawati et al., 2023) and contains diverse and useful bacteria (Hernandez et al., 2014). Alternatively, chicken manure also ameliorates soil quality and enhances crop performance. The dry forms of chicken manure and vermicompost contain higher quantities of nitrogen and phosphorous compared to other organic sources (Anandyawati et al., 2023). Research by Laleh et al. (2021) found that applying cow manure at 20 t ha^{-1} plus nitrogen at 100 kg ha^{-1} produced the highest biological yield, seed, and leaf extract from cannabis. The highest oil content was obtained by a maximum of 50 kg N ha^{-1}, without phosphorous. Thirty t ha^{-1} cow manure with 100 kg N ha^{-1} increased the leaf harvest index and decreased the seed harvest index. Nitrogen consumption also increased the seed oil content and yield. Phosphorus increased the biomass and extracts of seeds and leaves, as well as biological, seed, and oil yields (Laleh et al., 2021). In a field experiment, the positive effect of vermicompost on cannabis growth was greater than the effect of equivalent mineral fertilizer concentrations. When using vermicompost at 5 and 10 t ha^{-1}, plant growth increased by 40-60%, stem yield increased by 40%, and seed yield 20 t ha^{-1}. These results were derived under local agroclimatic conditions; thus it is reasonable to expect a significant increase in yield from the application of vermicompost (Stramkale et al., 2021). Meanwhile, little is known about chicken manure in the context of cannabis growth and secondary metabolites. Assessing the impact of organic fertilizers on cannabis cultivation thus depends on quantifying biomass, concentrations of CBD and THC, and accumulation of heavy metals in the florets. A positive correlation has been reported between CBD and THC concentrations and nitrogen, potassium, calcium, and magnesium (Veazie et al., 2021). During the cannabis flowering phase, phosphorous plays a significant role in inducing a higher number of buds per plant (Gorelick and Bernstein, 2017). Phosphorous is slowly absorbed and concentrated in the leaves and healthy plants commonly retain about 0.1-0.44% phosphorous in their leaves (Caplan et al., 2017b). This study aimed to (1) investigate the effect of vermicompost and chicken manure on the growth, cannabinoid content, and heavy metal accumulation of *C. sativa* KKU05, (2) to evaluate the effect of organic fertilizers compared to no fertilizer, and (3) to investigate the effect of inorganic fertilizer on *C. sativa* KKU05 growth parameters. The results of this study will help guide GAPs and promote organic systems in the application of fertilizers for cannabis production.

Results

Chemical properties of soil and organic fertilizer before planting

The experimental site was sandy loam soil with a weakly alkaline pH. Organic matter, available phosphorous, exchangeable potassium, magnesium, iron, zinc, total chromium, and cadmium were significantly higher in topsoil (Table 1). However, the

Table 1. Chemical properties of soil and organic fertilizers before planting *Cannabis sativa* KKU05.
Distinct letters in the row indicate significant differences according to the Least Significant Difference (LSD) test ($P \leq 0.05$). ^{ns}Not significant. *Significant at the 0.05 probability level. **Significant at the 0.01 probability level.

Soil depth	Chemical properties															
	pH	OM (%)	EC ($dS\ m^{-1}$)	N (%)	P ($mg\ kg^{-1}$)	K ($mg\ kg^{-1}$)	Ca ($mg\ kg^{-1}$)	Mg ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)	Cr ($mg\ kg^{-1}$)	As ($mg\ kg^{-1}$)	Cd ($mg\ kg^{-1}$)	Pb ($mg\ kg^{-1}$)
0-15 (cm)	5.97^{b}	0.89^{a}	0.04	0.04	406.25^{a}	122.28^{a}	341.24^{b}	36.28^{a}	55.69^{a}	25.83^{b}	0.61^{b}	3.43^{a}	7.60^{a}	1.88	0.98^{a}	2.52
15-30 (cm)	6.00^{a}	0.65^{b}	0.04	0.04	204.17^{b}	62.76^{b}	418.44^{a}	21.52^{b}	43.13^{b}	34.61^{a}	0.77^{a}	2.63^{b}	5.22^{b}	1.68	0.56^{b}	2.80
F-test	**	**	ns	ns	**	**	**	**	**	**	**	**	**	ns	*	ns
Organic fertilizer	pH	OM (%)	EC ($dS\ m^{-1}$)	N (%)	P ($mg\ kg^{-1}$)	K ($mg\ kg^{-1}$)	Ca ($mg\ kg^{-1}$)	Mg ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)	Cr ($mg\ kg^{-1}$)	As ($mg\ kg^{-1}$)	Cd ($mg\ kg^{-1}$)	Pb ($mg\ kg^{-1}$)
Chicken manure	6.75^{b}	14.51^{b}	0.92	0.20^{b}	1.92^{a}	0.61^{a}	7.40^{a}	1.79^{a}	1.76^{b}	0.10	128.74^{a}	1,197.00^{a}	19.07^{b}	7.15^{a}	1.80^{a}	10.14^{a}
Vermicompost	6.86^{a}	40.31^{a}	0.92	1.30^{a}	0.62^{b}	0.36^{b}	1.75^{b}	0.49^{b}	3.57^{a}	0.10	24.17^{b}	206.86^{b}	7.50^{d}	2.59^{b}	0.84^{b}	2.34^{b}
F-test	**	**	ns	**	**	**	**	**	**	ns	**	**	**	**	**	**

Table 2. Growth of *Cannabis sativa* KKU05 exposed to different fertilizer applications at 30 days after transplanting.

Fertilizer	Plant height (cm)	Leaf area ($cm^2\ plant^{-1}$)	SPAD	Stem circumference (cm)
No fertilizer	42.31^{c}	59,106.00^{c}	49.51^{b}	6.45
Chicken manure	48.03^{b}	69,971.00^{bc}	52.91^{b}	6.22
Vermicompost	50.52^{b}	93,804.00^{ab}	53.44^{ab}	6.58
Inorganic NPK	59.51^{a}	108,228.00^{a}	57.92^{a}	6.83
F-test	**	*	**	ns

Distinct letters in the row indicate significant differences according to the Least Significant Difference (LSD) test ($P \leq 0.05$). ^{ns}Not significant. *Significant at the 0.05 probability level. **Significant at the 0.01 probability level.

Table 3. Growth parameters at 60 days after transplanting *Cannabis sativa* KKU05 with different fertilizer applications.

Fertilizer	Plant height (cm)	Leaf area ($cm^2\ plant^{-1}$)	SPAD	Stem circumference (cm)
No fertilizer	88.94	380,733.00	46.70^{b}	6.80
Chicken manure	78.42	656,538.00	46.65^{b}	6.82
Vermicompost	94.22	751,056.00	49.28^{b}	6.81
Inorganic NPK	107.00	912,172.00	58.30^{a}	7.45
F-test	ns	ns	*	ns

Distinct letters in the row indicate significant differences according to the Least Significant Difference (LSD) test ($P \leq 0.05$). ^{ns}Not significant. *Significant at the 0.05 probability level.

Table 4. Yield components of *Cannabis sativa* KKU05 exposed to different fertilizer applications at harvest 90 days after transplanting.

Fertilizer	Leaf dry weight ($g\ plant^{-1}$)	Stem dry weight ($g\ plant^{-1}$)	Root dry weight ($g\ plant^{-1}$)	Florets dry weight ($g\ plant^{-1}$)
No fertilizer	53.50	36.48^{b}	29.55^{b}	127.14
Chicken manure	70.72	43.12^{b}	39.06^{a}	140.64
Vermicompost	109.90	53.66^{a}	42.63^{a}	211.86
Inorganic NPK	111.40	54.05^{a}	43.77^{a}	224.62
F-test	ns	**	*	ns

Distinct letters in the row indicate significant differences according to the Least Significant Difference (LSD) test ($P \leq 0.05$). ^{ns}Not significant. *Significant at the 0.05 probability level. **Significant at the 0.01 probability level.

Table 5. Major cannabinoids in *Cannabis sativa* KKU05 florets at harvest (90 days after transplanting) exposed to different fertilizer applications.

Fertilizer	THC (%)	CBD (%)	THC (g plant^{-1})	CBD (g plant^{-1})
No fertilizer	0.10^{d}	2.54^{c}	0.13^{b}	3.22^{c}
Chicken manure	0.12^{c}	3.20^{b}	0.23^{b}	5.95^{bc}
Vermicompost	0.21^{b}	4.91^{a}	0.31^{b}	7.44^{b}
Inorganic NPK	0.53^{a}	4.94^{a}	1.20^{a}	11.29^{a}
F-test	**	**	**	**

Distinct letters in the row indicate significant differences according to the Least Significant Difference (LSD) test ($P \leq 0.05$). **Significant at the 0.01 probability level.

Table 6. Fatty acid concentrations in *Cannabis sativa* KKU05 exposed to different fertilizer applications at harvest (90 days after transplanting).

Fertilizer	Omega 3 (%)	Omega 6 (%)	Omega 9 (%)
No fertilizer	14.35	47.51	13.80
Chicken manure	14.96	54.30	14.82
Vermicompost	17.19	54.88	16.68
Inorganic NPK	17.89	56.80	17.35
F-test	ns	ns	ns

Distinct letters in the row indicate significant differences according to the Least Significant Difference (LSD) test ($P \leq 0.05$). ^{ns}Not significant.

Table 7. Macronutrients and heavy metals in different parts of *Cannabis sativa* KKU05 grown under different fertilizer treatments.

Fertilizer	Leaf						
	N (%)	P (%)	K (%)	Cr (mg kg^{-1})	As (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})
No fertilizer	2.13^{d}	0.36^{b}	2.17^{d}	5.55^{a}	2.69^{c}	0.30^{ab}	0.70^{a}
Chicken manure	2.50^{a}	0.35^{c}	2.36^{b}	5.35^{c}	3.46^{b}	0.33^{a}	0.30^{b}
Vermicompost	2.37^{b}	0.38^{a}	2.45^{a}	5.47^{b}	3.74^{ab}	0.27^{b}	0.24^{b}
Inorganic NPK	2.31^{c}	0.36^{b}	2.22^{c}	5.60^{a}	4.26^{a}	0.30^{ab}	0.21^{b}
F-test	**	**	**	**	**	*	**
Fertilizer	Stem						
	N (%)	P (%)	K (%)	Cr (mg kg^{-1})	As (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})
No fertilizer	0.45^{a}	0.08^{a}	1.26^{a}	9.74^{a}	2.85^{c}	0.27	0.56^{a}
Chicken manure	0.42^{b}	0.06^{b}	1.20^{a}	8.37^{b}	3.23^{b}	0.27	0.39^{c}
Vermicompost	0.43^{b}	0.08^{a}	1.01^{b}	7.01^{c}	3.16^{b}	0.30	0.56^{a}
Inorganic NPK	0.40^{c}	0.06^{b}	1.00^{b}	8.42^{b}	3.85^{a}	0.27	0.45^{b}
F-test	**	**	**	**	**	ns	**
Fertilizer	Root						
	N (%)	P (%)	K (%)	Cr (mg kg^{-1})	As (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})
No fertilizer	0.96^{a}	0.26^{b}	1.30^{a}	10.86^{b}	3.58	0.33^{c}	0.33^{ab}
Chicken manure	0.80^{b}	0.23^{c}	1.28^{a}	9.75^{c}	3.55	0.39^{ab}	0.38^{a}
Vermicompost	0.78^{b}	0.21^{d}	0.95^{c}	11.09^{a}	3.23	0.42^{a}	0.21^{c}
Inorganic NPK	0.96^{a}	0.33^{a}	1.09^{b}	9.15^{d}	3.38	0.36^{bc}	0.27^{bc}
F-test	**	**	**	**	ns	**	*
Fertilizer	Florets						
	N (%)	P (%)	K (%)	Cr (mg kg^{-1})	As (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})
No fertilizer	3.14^{b}	0.68^{a}	2.06^{c}	6.94^{bc}	2.81	0.33	0.35
Chicken manure	2.98^{c}	0.60^{b}	2.10^{c}	7.11^{ab}	3.00	0.30	0.48
Vermicompost	3.21^{a}	0.55^{c}	2.29^{b}	7.16^{a}	3.35	0.33	0.44
Inorganic NPK	3.23^{a}	0.54^{d}	2.45^{a}	6.83^{c}	3.21	0.27	0.36
F-test	**	**	**	*	ns	ns	ns

Distinct letters in the row indicate significant differences according to the Least Significant Difference (LSD) test ($P \leq 0.05$). ^{ns}Not significant. *Significant at the 0.05 probability level. **Significant at the 0.01 probability level.

total concentration of heavy metals at this site was lower than what is within the normal range for plant growth, except for cadmium, which was 0.36 mg kg^{-1} (report the normal reference range here) (Kubier et al., 2019). The salinity of the site was lower than 2 dS m^{-1} and therefore considered to have no detrimental effect on cannabis growth and yield. Vermicompost had the highest pH, OM, total nitrogen, and iron levels (Table 1). In contrast, chicken manure showed the highest total phosphorous, potassium, calcium, magnesium, copper, zinc, arsenic, cadmium, and lead. Although chromium, arsenic, cadmium, and lead were found in both organic fertilizers, levels did not exceed the acceptable parameters set out by the Ministries of Agriculture for China, Canada, and the United States (Li et al., 2021).

Aboveground plant performance

Cannabis sativa KKU05 plants were observed at 30 days after transplanting (DAT). Growth characteristics of the plants, such as plant height, leaf area, and soil plant analysis development (SPAD) or chlorophyll content, showed significant differences among the three fertilizers used. The inorganic NPK fertilizer resulted in the highest plant height (59.51 cm), leaf area (108,228 cm^{2}), and SPAD (57.92). Differences in stem circumference were not significant among the fertilizers, but the application of inorganic NPK was associated with the highest stem circumference (Table 2), followed by vermicompost. Similar results were observed at 60 DAT; inorganic NPK fertilizer was associated with the highest SPAD. However, at 60 DAT, plant height, leaf area, and stem circumference were not significantly different among fertilizer types. Plant height, leaf area, and stem circumference tended to increase with fertilizer application, with inorganic NPK associated with the biggest increases, followed by vermicompost (Table 3). At harvest (90 DAT), stem and root dry weights, were significantly different among the fertilizers used. Inorganic NPK and vermicompost applications were associated with higher stem and root dry weights, with 54.05 stem g plant^{-1} and 43.77 root g plant^{-1} for NPK, and 53.66 stem g plant^{-1} and 42.63 root g plant^{-1} for vermicompost. Leaf and floret dry weights did not differ significantly, but the highest floret dry weight was found with NPK fertilizer (Table 4), followed by vermicompost. The application of inorganic NPK and vermicompost was associated with higher floret biomasses of 224.62 and 211.86 g plant^{-1}, respectively.

Cannabinoid and fatty acid concentrations

Cannabis sativa KKU05 produced both CBD and THC, even when no fertilizer was applied. Plants treated with NPK had the highest concentrations of CBD (4.94%) and THC (0.53%). In contrast, plants treated with no fertilizer showed lower concentrations of CBD and THC (Table 5). Vermicompost was associated with 4.91% CBD and higher levels of omega 3 and 6, compared to the no fertilizer group (Table 6). However, the differences in essential fatty acid levels among the fertilizer groups were not statistically significant.

Nitrogen, phosphorous, potassium, and heavy metals

Nitrogen, phosphorous, and potassium levels at harvest were significantly higher in the florets than in the leaves, roots, and stems of *C. sativa* KKU05 across all fertilizer treatments. Applying vermicompost was associated with increased foliar phosphorous and potassium concentrations. Conversely, lead, arsenic, and cadmium levels in the cannabis roots, leaves, stems, and florets varied amongst the different fertilizers. Exceeded cadmium range in soil (0.36 mg kg^{-1}) may affect plant growth and yield quality (Kubier et al., 2019). However, the different fertilizers did not appear to exert a significant effect on floret cadmium levels (Table 7).

Discussion

Our study is the first to demonstrate the effectiveness of chicken manure in enhancing bio-active compounds of *C. sativa* KKU05 in comparison to vermicompost and inorganic NPK fertilizer. After 30 DAT, the plants were receiving fewer than 12 h of daily light exposure and thus began to develop reproductive rather than vegetative growth, and photosynthesis activity shifted from the plants' leaves and stems to the floret. This shift causes the leaves to fall until the harvest stage. While cannabinoid compounds are found throughout a cannabis plant, they tend to accumulate at their highest concentrations in the floret (Potter, 2014). While *C. sativa* KKU05 can grow without added fertilizer, our results indicate that floret biomass and cannabinoid concentrations benefit from fertiliser application.

Multiple studies indicate that the metabolism of phytocannabinoids is highly sensitive to mineral (Bernstein et al., 2019; Malík et al., 2021) and organic nutrition (Caplan et al., 2017a; Caplan et al., 2017b; Bruce et al., 2022) with a positive correlation between inorganic fertilizer application and floret yield. Our results similarly show a positive correlation between floret biomass and cannabinoid concentrations with both inorganic NPK and vermicompost. However, abiotic stress like nutrient deprivation can trigger the production of secondary metabolites vital for plant self-defence and survival (Aguirre-Becerra et al., 2021). Saloner and Bernstein (2021) found increased terpenoids and cannabinoids associated with nitrogen deprivation. Higher efficiency of nitrogen, phosphorous, and potassium utilization by nutrient-deprived plants, characterized by larger mobilization and translocation of the nutrients, was observed. The agronomic efficiency of CBD yield for nitrogen and phosphorous increased by 34% with the organic fertilizers and 72% with the mineral fertilizers (Massuela et al. 2023). In addition, under nutrient deprivation, plant elicitors and stressors can produce metabolic responses (eustress) that alter signalling pathways and favour the synthesis of terpenoids and phenolic compounds (Aguirre-Becerra et al., 2021). Moreover, the application of inorganic NPK and vermicompost fertilizers resulted in higher levels of omega 3, 6, and 9. Our results are similar to Caplan et al.'s (2017b), who also found that organic fertilizers increase cannabis yield. Other studies have also indicated positive effects of organic fertilizers on the quantitative and qualitative yields of medicinal plants (Laleh et al., 2021). Our results indicate that *C. sativa* KKU05 floret biomass and cannabinoid concentration respond to additional nitrogen in the context of acid sandy low-fertility soil. However, the form of nitrogen provided (ie. NH^{+}_{4} or NO^{-}_{3}) may exert an effect on the physiological function and biosynthesis of cannabinoids. Previous research has found higher NO^{-}_{3} in vermicompost while NH^{+}_{4} was higher in chicken manure (Huang et al., 2016).

At the same time, higher nitrogen in the florets when vermicompost or inorganic NPK fertilizers are utilized, could result in higher concentrations of cannabinoid compounds than when chicken manure or no fertilizer is used. There was no detected fertiliser effect on concentrations of omega 3 6 and 9 fatty acids. In the Saloner and Bernstein study (2022), the experimental planting area was treated with 33 t ha^{-1} of cow manure so that the *C. sativa* KKU05 could grow without added fertilizer; the accumulation of nitrogen in the stems was significantly higher than with other fertilizer applications. Where there is sufficient nitrogen in the soil but a deficit of phosphorous, plants will accumulate an excess of nitrogen in their stems because, in the absence of phosphorous, they are unable to utilize the nitrogen to build organic compounds, a process called synergism (Blandino et al., 2023). In this study, arsenic, cadmium, and lead accumulated more in the plants' leaves, roots, stems, and florets with the application of inorganic NPK, vermicompost, chicken manure, and non-fertilizer, respectively. It is worth noting that, in the absence of soil or water contamination, heavy metals are found at the highest concentrations in inorganic NPK, followed by chicken manure, and vermicompost. However, in this study, fertilizer choice was not associated with significant increases to heavy metals in the florets. Cannabis roots accumulated higher amounts of cadmium compared to the leaves, flowers, and stems. Golia et al. (2023) reported that cadmium accumulation has no observed negative effects on cannabis germination but hinders photosynthetic capacity and hence may reduce future yields. At the same time, *C. sativa* KKU05 leaves, roots, and stems appear to accumulate more heavy metals with shorter life cycles under field conditions. These characteristics of *C. sativa* KKU05, together with a relatively deep root system and efficient translocation of contaminants to the shoots, highlight KKU05 as a potential natural hyperaccumulator of dangerous heavy metals in contaminated areas. As chicken manure is higher in NH^{+}_{4} and heavy metals zinc (1,197 mg kg^{-1}), copper (128.74 mg kg^{-1}), chromium (19.07 mg kg^{-1}), lead (10.14 mg kg^{-1}), arsenic (7.15 mg kg^{-1}), and cadmium (1.80 mg kg^{-1}) compared to vermicompost, further research is needed to reduce heavy metal accumulation in cannabis production. Vermicompost as a source of organic nitrogen and phosphorous for *C. sativa* KKU05 production should be considered as a means of reducing the use of inorganic KNP fertilizer without sacrificing floret biomass and cannabinoid yields. However, organic fertilizers have some limitations. The nutrient concentrations in organic fertilizers are lower than for inorganic KNP, thus requiring application of larger volumes, and the release of nutrients is slower from organic fertilizers, which slows the crop's initial growth stage. In contrast, inorganic NPK dissolves rapidly and releases greater amounts of macronutrients readily absorbed by the plants. Our findings suggest that the most benefit could be gained from mixing vermicompost together with inorganic NPK, rather than using a single organic or inorganic fertilizer product.

Materials and methods

Seedling preparation

The *C. sativa* KKU05 hybrid seeds were soaked in warm water at 40°C overnight and then washed three times with sterile water. The seeds were then cultured on germination paper for two days. The sprouts with soft roots were transferred to peat moss soil in 104-well trays, kept in greenhouse conditions for two weeks, and watered every 2-3 days. To induce a strong root system, the seedlings were then transferred into 10 cm pots for three weeks before being transplanted into the experimental field site.

Experimental setup and crop management

The experiment was conducted at the Cannabis Research Institute, Khon Kaen University, Khon Kaen (16° 25.00′ N, 102° 50.00′ E), Thailand, at the beginning of the 2022 and 2023 rain seasons. The rain season is May to October and is followed by the cool and dry seasons. Over the crop growth periods in 2021 and 2022, rainfall was 750.78 and 982.47 mm, respectively. The maximum recorded temperatures were 33.23 and 32.72 °C, and the minimum temperatures 23.62 and 23.58 ºC in 2021 and 2022, respectively. For field preparation, the soil was ploughed and left to dry for one week. Planting plots were then measured with a planting distance of 160 × 100 cm. The soil was ploughed a second time and a trench measuring 1 m wide, 60 cm high, and 30 m long was raised for 16 plots. Soil pH was adjusted with 100 kg of cow manure $plot^{-1}$ and lime at 312.50 kg ha^{-1}. To prevent weeds, planting plots were covered with plastic and black plastic net along the aisle of each plot. Wooden posts and a drip irrigation system were then installed. As *C. sativa* is a short-day plant, light emitting diodes (LEDs) were installed for one month to enhance vegetative growth after transplanting.

Before planting, soil samples were collected at a depth of 0-15 and 15-30 cm from ten points $plot^{-1}$ (n=10), mixed, and preserved in plastic bags for analysis of chemical properties. Soil pH was measured with a pH meter, organic matter (OM) by the Walkley and Black method (Walkley and Black, 1947), electrical conductivity (EC) by EC meter, total nitrogen (N) by the Kjeldahl method (Bremner, 1965), available phosphorous (P) by the Bray II and molybdenum-blue method (Bray and Kurtz, 1945), exchangeable potassium (K), calcium (Ca), and magnesium (Mg) by 1 N NH_4OAc, pH 7, and flame photometry methods, iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) by diethylenetriaminepentaacetic acid (DTPA) pH 7.3 extraction, atomic absorption spectrophotometry, and total chromium (Cr), arsenic (As), cadmium (Cd) and lead (Pb) by wet digestion and inductively coupled plasma optical emission spectroscopy (ICP-OES). To measure soil nutrients, air-dried soil samples were crushed and passed through a 0.25 mm sieve.

Planting and fertilizer treatments

The hemp seedlings were transplanted into the field at 40-45 days after germination with a sowing spacing of 160 × 100 cm for 30 plants $plot^{-1}$. Drip irrigation was carried out daily for 30 min. The trial was carried out in a randomized complete block design with four treatments: (T1) no fertilizer; (T2) chicken manure at 6,250 kg ha^{-1} (12.50 kg N ha^{-1}, 120 kg P ha^{-1}, and 38.13 kg K ha^{-1}); (T3) vermicompost at 6,250 kg ha^{-1} (81.25 kg N ha^{-1}, 38.75 kg P ha^{-1}, and 22.50 kg K ha^{-1}); and (T4) inorganic KNP fertilizer (50 kg N ha^{-1}, 232 kg P ha^{-1}, and 170 kg K ha^{-1}). Fertilizer was applied during the vegetative and reproductive growth stages. The chemical properties of the vermicompost and chicken manure were analysed prior to initiation of the trial. The pH was determined using a pH meter, OM was measured by the Walkley and Black method (Walkley and Black, 1947), and electrical conductivity (EC) was measured using an EC meter. Total nitrogen was measured using the Kjeldahl method (Bremner, 1965), total phosphorous was measured using Wet digestion (Nitric perchloric) Spectrophotometer, and total K, Ca, Mg, Fe, Mn, Cu, and Zn were measured using Wet digestion (Nitric perchloric) atomic absorption spectrophotometry. Total Cr, As, Cd, and Pb were measured using Wet digestion (Nitric perchloric) ICP-OES.

Sample and data collection

During the 2022 and 2023 growing seasons, stem height was measured for ten KKU05 hybrid plants plot^{-1}. The SPAD value was determined using a chlorophyll meter model SPAD-502Plus, China. One-to-two months after transplanting, leaf area was measured with a leaf area meter, model LI-3100C (LI-COR Inc, Lincoln, Nebraska, USA), and stem circumference was measured at 12 cm above the ground using a vernier calliper. At the harvest stage three months after transplanting, ten KKU05 hybrid plants plot^{-1} were harvested and incubated in a greenhouse at 40 °C for 1-2 days. After incubation, the ten plants from each plot were separated into floret, roots, stems, and leaves. The florets were transferred to a hot air oven at 40 °C for 48 h to achieve a constant dry weight and measure their total biomass. The roots, stems, and leaves were each transferred to a hot air oven at 80 °C for 48 h to achieve a constant dry weight for measurement of their total biomass. The dried florets, roots, stems, and leaves were then ground into powder and analysed for total nitrogen by the Bremner and Mulvaney method (Bremner and Mulvaney, 1982), total phosphorous by Wet digestion and Spectrophotometry (Hesse, 1971; Barton, 1948), and potassium content by wet digestion (nitric perchloric) and atomic absorption spectrophotometry in the leaf. Heavy metals such as arsenic, cadmium, chromium, and lead were analysed using wet digestion and inductively coupled plasma optical emission spectroscopy (ICP-OES), Plasma Quant 9000, 13-58500-AQ121, Analytik Jena Far East (Thailand) Ltd. Germany. Concentrations of omega 3, 6, and 9 fatty acids, THC, and CBD were also determined.

Analysis of cannabinoids

Five grams of dried florets from each of the four treatment arms were powdered and heated at 100°C for 3 h. To extract the cannabinoids, 1 g of the sample was mixed with 20 ml of hexane and kept in an incubator shaker at 28°C and 120 rpm for 24 h. To obtain the crude cannabinoid extract, the solvent fraction was filtered and evaporated using a vacuum funnel (Buchner funnel, Fisher Scientific, Sweden) and a rotary evaporator (Rotavapor R-300, Buchi, Switzerland). High-performance liquid chromatography (HPLC) (Alliance HPLC System, Waters, USA) was used to quantify the crude extract. Differentiation of CBD and THC in the crude cannabinoid extract was achieved by comparing the retention times to known chromatograms of the standard compounds. Extrapolating peak areas of the target compounds with the calibration curves was performed to quantify the relative concentrations of cannabinoid metabolites (Zivovinovic et al., 2018). Chromatographic separation was performed on a CORTECS Shield RP18 column (2.7 µm particle size, 4.6 mm × 150 mm) with a column temperature of 35°C. The mobile phase was composed of 0.1% Trifluoroacetic acid (TFA) in both water and acetonitrile under isocratic elution in a 40:60 (v/v) ratio run at a 1.5 ml min^{-1} flow rate. A hydrophilic PTFE membrane filter (0.5µm, ADVANTEC, Toyo Roshi Kaisha, Ltd. Tokyo, Japan) was used to filter the mobile phase solution. A photodiode array (PDA) detector at 228 nm was used for monitoring the analyte.

Analysis of omega 3, 6, and 9 fatty acids

Cannabis seed oil was obtained by extracting 1 g of seed with 5 ml of hexane at room temperature (25±2 °C) for 12 h. Whatman filter papers were used to remove trace amounts of seed from the oil. The filtered oil was then stored in amber bottles with N_2 gas at 4°C. The methylation reaction was carried out by mixing 500 µg of cannabis seed oil with 1 ml Ruiz's solution (methanol: toluene: 2,2-dimethoxy propane: H_2SO_4 = 60: 20: 5: 2) and 500 µl heptane. This mixture was then stored at 80°C for 2 h. Fatty acid composition was determined by GC (Agilent Technologies, model GC7890, CA, USA) equipped with an autosampler (model 7693), a flame ionization detector (FID), and a Stabilwax®-MS column (30 m × 250 µm × 0.25 µm). The column temperature started at 210°C for 5 min, and a heating ramp was set at 20°C min^{-1} until 230°C was reached for 4 min. The carrier gas was helium at a flow rate of 1 ml min^{-1}.

Statistical analysis

We employed analysis of variance (ANOVA) to evaluate differences in mean outcomes among the four fertilizer treatments and two crop cycles. If a significant effect was detected, the least significant difference (LSD) test at a probability level of 0.05 was conducted. Statistical analysis was carried out using the Statistics version 10.0 software.

Conclusions

Inorganic NPK fertilizer application promoted plant height and leaf growth between 30 and 60 days after transplanting (DAT). However, this application did not significantly affect cannabis inflorescence development compared to vermicompost. However, inorganic NPK was associated with higher Δ*9*-tetrahydrocannabinol (THC) and cannabidiol (CBD) concentrations in the florets of *Cannabis sativa* KKU05. Of the two organic fertilizers tested, vermicompost was associated with higher concentrations of THC and CBD, at 0.21% and 4.91%, respectively. Fertilizer type was also associated with varying degrees of heavy metal accumulation in different parts of the plant, and the highest heavy metal levels were observed in the plants fertilised with inorganic NPK. Overall, the roots accumulated more heavy metals than the leaves, stems, or florets. The results of this experiment suggest that organic fertilizer should be used in combination with inorganic KNP fertilizer to enhance cannabis production under Good Agricultural Practices (GAP). Vermicompost mixed with inorganic NPK is a promising approach to optimizing *C. sativa* quality and yields, while also sustaining the long-term use of synthetic fertilizers.

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Author contribution

Conceptualization: K.S., C.L. Methodology: K.S., C.L. Validation: K.S. Formal analysis: K.S., T.S., J.P., K.L. Investigation: K.S., T.S. Resources: K.S., C.L., J.P., K.L. Data curation: K.S., T.S., J.P., K.L. Writing-original draft: K.S. Writing-review & editing: K.S., J.P., K.L Supervision: K.S. Project administration: K.S. Funding acquisition: K.S. All co-authors reviewed the final version and approved the manuscript before submission.

Conflict of interest

The author(s) declare no conflict of interests.

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