



Asian Journal of Crop Science

ISSN 1994-7879

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Research Article

Influence of Temperature on *Cannabis sativa* Seed Germination: Insights from Lusikisiki Ecotypes, Eastern Cape

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Abstract

Background and Objective: Temperature is considered a key environmental factor that influences germination, as it plays a critical role in breaking dormancy. Additionally, the successful establishment of seedlings is largely dependent on their germination potential. This study, investigated the effects of varying low temperatures, on the seed germination of medicinal *Cannabis sativa* ecotypes collected in Lusikisiki, Eastern Cape Province. **Materials and Methods:** Seeds of medicinal cannabis were collected in the wild from three villages namely: V1, V2 and V3 in Lusikisiki and were subjected to varying minimum temperatures viz: 8, 10 and 12°C. Thirty-five seeds per village were placed in 9 × 1.5 cm Petri dishes lined with moistened double-layer filter papers. Petri dishes were placed in a growth chamber (Labex, Scientific, Model 357, EL, SA) set at different temperature levels for fourteen days. At two days and daily until 14 days after sowing (DAS) parameters namely, number of seeds germinated, days to 50% (T50) germination, final germination percent (FGP), coefficient of velocity of gemination (CVG), mean gemination time (MGT) and the length of hypocotyl and radicle were determined. **Results:** *Cannabis sativa* seeds begin to sprout at 5 days and the germination halts at 13th days DAS irrespective of temperature level and seed origin. About 12°C had the highest number of germinated seeds and 8°C recorded as the lowest. The V3 seeds had the highest germination proportion and V1 had the lowest. The V3 seeds demonstrated a good germination potential in all indices tested. The longest radicle was recorded in V3 at 12°C and the equal hypocotyl length was obtained at 12°C irrespective of village. **Conclusion:** This study suggested that cannabis sown at 12°C had a rapid germination and the seeds collected from areas of high geographic altitudes could be a preferred choice for cannabis production as they show adaptability to varying minimum temperatures.

Key words: *Cannabis sativa*, seed germination, seedling development, phytochemical composition, Lusikisiki

Citation: Dumani, A., I. Egbichi, A.O. Oyedeji, T.T. Silwana, B. Mpambani and H.A. Seepe, 2024. Influence of temperature on *Cannabis sativa* seed germination: Insights from Lusikisiki ecotypes, Eastern Cape. Asian J. Crop Sci., 16: 6-21.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cannabis sativa commonly known as Marijuana or Dagga is a member of the cannabis plant family and is the oldest herb known for centuries in Southern Africa for its medicinal purposes¹. Despite its multiple use the cultivation and use of this plant was declared illegal in South Africa in 1928 due to its psychotic properties². The medications derived from this plant play a crucial role in the treatment of conditions such as cancer, Alzheimer's, multiple sclerosis and chronic pains³. This is attributed to its historical legal status of being prescribed as a schedule 1 drug category plant⁴. The medicinal significance of *Cannabis sativa* has prompted its legalization globally⁵. The recent changes in this plant's legality, regulations and commercialization for its industrial, medicinal and recreational use have stimulated interest in its cultivation, processing and trading⁶. Although cannabis has a vast history in human culture with great medicinal and economic potential, it remains a poorly understood agricultural crop^{7,8}. There is insufficient or limited information on the agronomic protocols, seed germination and phytochemical composition of *Cannabis* materials (seeds, flowers and leafy parts) connected at various locations⁹. The current renaissance and the increasing demand for Cannabis products for medicinal and economic potential have triggered researchers to study its growth requirements^{1,10}. In plant production, seed germination is the first physiological stage in the plant life cycle¹¹. Seed germination is defined as the ability of the seed to sprout or emerge and develop into a healthy seedling^{12,13}. Furthermore, seed germination is a biological process that is influenced by the genotype and other physical factors such as medium composition, environment as well as climatic conditions¹⁴. Temperature is considered as the key environmental factor that influences germination, as it plays a critical role in breaking dormancy¹⁵. In addition, the successful establishment of seedlings is largely dependent on their germination potential¹⁶. Temperature affects both the seed germination percentage as well as the germination rate¹⁷. Thus, seed germination response to temperature is imperative for most crops of economic value¹⁸. Hence, it is considered an important environmental factor when planning and preparing for a planting time/season¹⁰. The minimum temperature is the lowest temperature at which the seed germination can occur, while the maximum temperature is the highest temperature at which the seed can germinate¹⁹. Moreover, beyond the maximum temperature, seeds get damaged, thermally inhibited from germination and even enter secondary dormancy²⁰. Consequently, there are three ways in which the temperature acts to regulate germination, (i) It determines the capacity and the

germination rate, (ii) Reduces primary and secondary dormancy and (iii) Encourages secondary dormancy²¹. The current agricultural challenges due to climate variability and change necessitate the understanding of medicinal cannabis seed germination over a range of low temperatures.

Numerous studies and crop models developed for industrial cannabis (hemp) production elsewhere in the world, suggest that its germination is faster when minimum soil temperatures are between 13 and 18.5°C²². However, several studies suggest that the minimum temperature for hemp germination is 10°C^{18,23,24}. Understanding the cultivation systems of this plant is essential to ensure quality produce and yields for enhanced production of medicinal and pharmaceutical products²⁵. There is limited or no literature on the influence of temperature on the time to initial germination of medicinal cannabis (*Cannabis sativa*) in South Africa. Subsequently, a better understanding of the germination process of this crop may improve its cultivation conditions as well as the produce quality to ensure constant supply for sustainable product development. Hence, this study aims to evaluate the effect of temperature on seed germination of *Cannabis sativa* collected in Lusikisiki.

MATERIALS AND METHODS

Study location

Collection of *Cannabis sativa* seeds: One thousand seeds per village of medicinal cannabis (*Cannabis sativa*) were collected in the wild within the three villages where the cannabis grows in abundance at Lusikisiki in Ingquza Hill Local Municipality or Tambo District Municipality in the Eastern Cape Province, South Africa. Due to current legal status and implications in South Africa, the names of the villages are withheld and as such simply referred to as village 1, 2 and 3, respectively, to protect their identities. These villages are situated in the Indigenous valley forests alongside the riverbanks and are characterized by different geographic altitudes as well as different slope orientations. Thus villages 1 and 3 are the North-facing slopes while village 2 is the South-facing slope. They are situated at the geographic altitudes of 200, 300 and 800 m above sea level (ASL), respectively.

Experimental design and procedure

***Cannabis sativa* seeds germination:** The *in vitro* seeds germination experiment was conducted in November, 2023 and validated in January, 2024 in the Agronomy Laboratory at Döhne Agricultural Development Institute (32°39'17"S and 27°29'46"E) in Stutterheim, Eastern Cape Province, South Africa. This experiment was conducted under controlled conditions of 24 hrs darkness with temperature levels set

accordingly in a growth chamber (Labex, Scientific, Model 357, EL, SA) for 14 days. A factorial experiment was laid out in a Complete Randomised Design (CRD) with three replicates. Seeds from different localities were the main plots while the subplots were the three different minimum temperature levels namely: The 8°C below the minimum, 10°C minimum and 12°C above the minimum. Prior experiment, petri dishes 9×1.5 cm were disinfected using hypochlorite with distilled water and were allowed to dry before use. Double-layer Whatman no. 1 filter papers were placed inside each Petri dish. Before sowing a seed viability pre-test was conducted whereby the seeds were subjected to a water test and the floating seeds were discarded and regarded as unviable whereas the sinking/remaining seeds were regarded viable and were disinfected with ethanol and allowed to dry as per²⁶. At sowing three replicates of 35 seeds of *Cannabis sativa* per village were placed in a Petri dish lined with moistened double-layer filter papers. After sowing the Petri dishes were placed in a growth chamber (Labex, Scientific, Model 357, EL, SA) set at different minimum temperature levels in a constant circle of 24 hrs light darkness for 14 days²³. The doses for temperature treatments were based on the available literature on related crops such as industrial cannabis (hemp) as no research data is found on medicinal cannabis¹⁸.

Data collection: At 2 days and daily until 14 days after sowing (DAS), the following germination data was recorded:

- Number of seeds germinated was determined through count
- Number of days to 50% (T50) emergence was determined using the equations by Coolbear *et al.*²⁷ modified by Raphael *et al.*²⁸:

$$t_{50} = T_i + \frac{(N + \frac{1}{2} - N_i)(T_j - T_i)}{N_i - N_j}$$

Where:

t50 = Median germination time

N = Final number of germinated seeds

N_i and N_j = Total number of seeds germinated in adjacent counts at time T_i and T_j, respectively

When,

$$N_i < N + \frac{1}{2} < N_j$$

- Variables related to germination and initial seedling growth such as the coefficient of velocity of germination (CVG) and mean germination time (MGT) were determined using the following equations:

$$CVG = \frac{\sum N_i}{N_i T_i} \times 100$$

Where:

N = Number of seeds germinated on day

i and T = Number of days from seeding corresponding to N²⁹:

$$MGT = \frac{\sum (n \times d)}{N}$$

Where:

n = Number of seeds germinated on each day

d = Number of days from the beginning of the test

N = Total number of seeds germinated at the termination of the experiment³⁰. At 14 days the experiment was terminated

- The cumulative data was used to calculate the final germination percent (FGP) using the equation by Okello *et al.*³¹:

$$FGP = \frac{N_g}{N_t} \times 100$$

Where:

N_g = Number of germinated seeds

N_t = Total number of seeds

- Then the length of both hypocotyl and radicle was measured using a centimeter (cm) ruler

Statistical analysis: Collected germination data was analyzed using SAS statistical software version 9.4. A Two-way Analysis of Variance (ANOVA) was performed for each parameter and treatment using the General Linear Model procedure (PROC GLM) of SAS statistical software. Fisher's Least Significant Difference (LSD) (0.05) values were calculated at the p = 0.05 confidence level.

RESULTS

Effect of different temperature levels on seed germination potential of *Cannabis sativa*: Results on the effect of temperature (°C) on the number of seeds germinated were

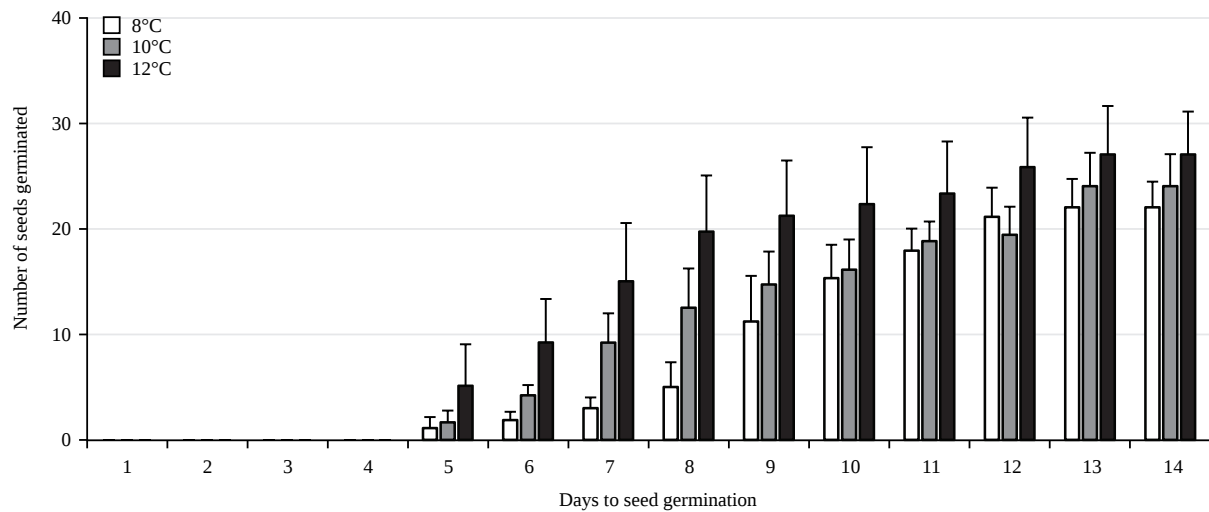


Fig. 1: Effect of low temperature ($^{\circ}\text{C}$) levels on the number (count) of seed germinated of *Cannabis sativa* collected in different localities at Lusikisiki
Bars bearing the same height indicate no significant differences ($p = 0.05$)

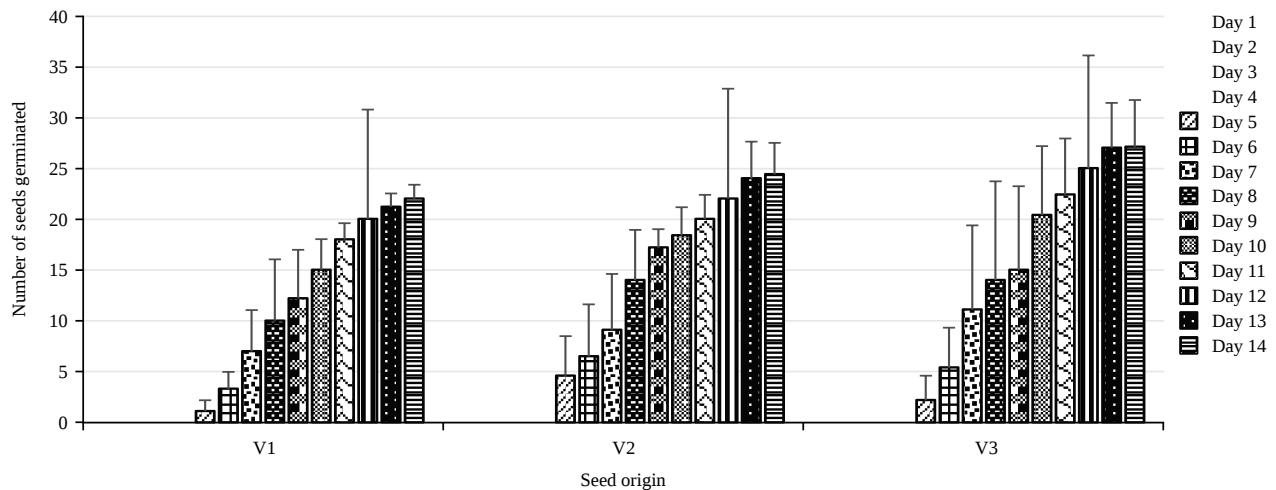


Fig. 2: Effect of the seed origin on the number of *Cannabis sativa* seeds germinated
Bars bearing the same height indicate no significant differences ($p = 0.05$)

presented (Fig. 1). The results revealed that inducing the low temperature levels had a highly significant ($p < 0.00$) influence on the number of seeds germinated of *Cannabis sativa*. The first seed sprout (protrusion) was observed on the 5 days after seed sowing (DAS) in all the treatments with the highest mean obtained in seeds that were exposed in 12°C (5.11) followed by 10°C (1.66) and the lowest seed sprouting was obtained in seeds sown at 8°C throughout the experiment. On the eight DAS, the seeds induced at 12°C (19.7) recorded the highest number of germinated seeds and lower germination was obtained at 8°C (5) and the trend was observed throughout

until the experiment was terminated at fourteen DAS. The maximum seed sprout was obtained on the thirteenth DAS in all treatments and was steady until the experiment was terminated at fourteen DAS, whereby the highest germination mean was attained in seeds sown at 12°C (27) while the lowest was recorded at 8°C (22).

Effect of seed origin on seed germination potential of *Cannabis sativa*: The results show that the number of seeds germinated (sprouting) was highly significantly ($p < 0.00$) influenced by the seed origin (Fig. 2). The first seed

Table 1: Interactive effect of seed origin/village and temperature levels on the number of days to seed germination of *Cannabis sativa*

Treatment	Days to seed germination										
Seed origin × Temp (°C)	Day 1-4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
V1 × 8	0	0.66 ^c	1.3 ^e	1.3 ^{dd}	2.7 ^f	7.3 ^e	13.7 ^{ed}	17 ^c	21 ^{cb}	21 ^c	21.3 ^c
V2 × 8	0	1.3 ^c	1.7 ^{de}	3 ^d	7.3 ^{de}	15.7 ^{bb}	16 ^{ced}	17.6 ^c	20.3 ^{cd}	21 ^c	22 ^c
V3 × 8	0	1.3 ^c	2.7 ^{dce}	3 ^d	5 ^{fe}	10.7 ^{ed}	16.3 ^{ced}	19 ^c	22 ^{cb}	23 ^c	23 ^c
V1 × 10	0	0.66 ^c	3.7 ^{dce}	9.7 ^c	9.7 ^{dc}	11.3 ^{cd}	12.6 ^e	17 ^c	17 ^d	21 ^c	21.3 ^c
V2 × 10	0	3 ^{cb}	5 ^c	8.7 ^c	17 ^b	17.7 ^b	18.7 ^{cb}	19.6 ^{cb}	21 ^{cb}	23 ^c	23 ^c
V3 × 10	0	1.3 ^c	4 ^{dc}	9.3 ^c	11 ^c	15 ^b	17 ^{cbd}	19.6 ^{cb}	21 ^{cb}	27 ^b	27 ^b
V1 × 12	0	2 ^{cb}	4.7 ^c	8.7 ^c	16.3 ^b	18 ^b	18.3 ^{cb}	19 ^c	21.3 ^{cb}	22 ^c	23 ^c
V2 × 12	0	9.3 ^a	13 ^a	15.3 ^b	16.6 ^b	18.3 ^b	20.6 ^b	22.3 ^b	25 ^b	27.3 ^b	28 ^b
V3 × 12	0	4 ^b	10 ^b	21 ^a	26 ^a	27.3 ^a	28 ^a	28.7 ^a	31 ^a	31 ^a	31.3 ^a
Mean	-	2.63	5.11	8.9	12.4	15.7	17.9	20.1	22.4	24	24.5
CV (%)	-	57	26	18	15	13.5	13	7.8	9.4	8	6.7
p-value	-	0.01	0.001	0.002	0.002	0.001	0.04	0.005	0.03	0.07	0.04

Values in a column followed by a different letters are significantly different at $p \leq 0.05$, p-value: Probability value and CV (%): Coefficient of variance

protrusions were recorded on the 5 days after seed sowing (DAS) in all the treatments with the highest mean obtained on seeds collected at village two (V2) (4.6) followed by village three (V3) (2.2) and the lowest was recorded in village one (V1) (1.11). However, on the seventh DAS a slight increase in the number of germinated seeds was recorded at V3 (11.1) being the highest, followed by V2 (9.1), while the lowest number of germinated seeds was recorded at V1 (7) and were all significantly different to others. A significant similar trend continued until the thirteenth and fourteenth DAS when the germination reached steadiness with the highest seed germination recorded in V3 (27.1) followed by V2 (24.4) and the lowest (22) was attained in seeds collected from V1 and were all significantly different to each other.

Interactive effect between seed origin and temperature levels on seed germination potential of *Cannabis sativa* ecotypes:

The results showed that the interaction between seed origin and temperature levels had significantly ($p < 0.01$) influenced the number of days to seed germination except for the 13 days after sowing (DAS) which was not significantly different (Table 1). The first seed germination was observed in five DAS irrespective of treatment. With regards to seeds sown at 8°C temperature level: The significant ($p < 0.01$) first seed protrusion was observed on the five DAS with the highest number of germinations recorded on seeds collected from V2 (1.3) and V3 (1.3) equally and the lowest were obtained on seeds collected in V1 (0.66) and were not significantly different from each other. A similar trend was observed on six and seven DAS, respectively. However, significant seed germination was observed on the ninth DAS with the highest germinated seeds attained on seeds from V2 (15.7) followed by V3 (10.7) and the lowest was recorded on seeds collected at V1 (7.3) and were significantly different from each other.

At ten DAS a significant increase in a number of germinated seeds was recorded in all treatments, with the highest obtained in V3 (16.3) and V2 (16) and the lowest was recorded from seed collected in V1 (13.7) which was significantly different to others. A similar trend was observed until the fourteenth DAS when the experiment was terminated where the seeds from V3 (23) exhibited the highest germination potential closely followed by V2 (22) and the lowest was attained at V1 (21.3), respectively.

Considering the seed sown at 10°C, the highest number of seed germinated was attained on V2 (3) and was significantly different from all others, followed by V3 (1.3) and the lowest was recorded on seed collected at V1 (0.66). A similar trend was observed on seven until the eleventh DAS, respectively. A gradual increase was recorded on the eleventh and twelfth DAS when an equal number of germinated seeds was recorded at V2 (19.6) and V3 (19.6), respectively. However, a significant ($p < 0.04$) increase in a number of seeds germinated was observed on the thirteenth and fourteenth DAS with the highest number recorded on V3 (27) followed by V2 (23) and the lowest number was attained on seeds of V1 (21.3) and were significantly different to each other. Regarding the seeds sown at 12°C temperature level, seeds collected from V2 exhibited the highest number (9.3) (13) of germinated seeds followed by V3 (4) (10) and V1 had the lowest germination (2) and (4.7) on both the fifth-sixth DAS, respectively and were significantly different to each other. However, on the seventh DAS the highest number of germinated seeds was obtained in V3 (21) followed by V2 (15.3) and the lowest number was attained in V1 (8.7) collection and were all significantly different from each other. A similar trend was observed until fourteenth DAS with the seeds collected at V3 (31.3) recording the highest number of germinated seeds followed by V2 (28) and the lowest recorded

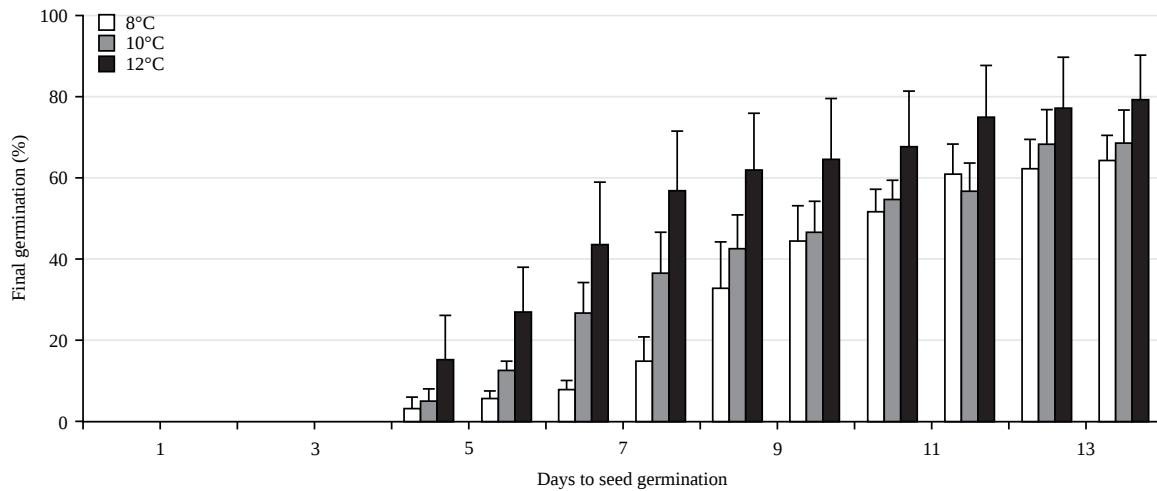


Fig. 3: Temperature effect on final germination percentage (FGP %) of *Cannabis sativa* seeds

Bars bearing the same height indicate no significant differences ($p = 0.05$)

Table 2: Interactive effect between seed origin/village, temperature level and the number of days to 50% seed germination of *Cannabis sativa*

Treatment	Means
Seed origin $\times$ Temp ($^{\circ}\text{C}$)	Days to 50% germination
V1 $\times$ 8	11.6 ^{ba}
V2 $\times$ 8	10.3 ^{bcd}
V3 $\times$ 8	10.6 ^{bc}
V1 $\times$ 10	12.3 ^a
V2 $\times$ 10	8.6 ^e
V3 $\times$ 10	10.3 ^{bcd}
V1 $\times$ 12	10 ^{ecd}
V2 $\times$ 12	9 ^{ed}
V3 $\times$ 12	7 ^f
Mean	10
CV (%)	8.9
p-value	0.02

Values in a column followed by the different letters are significantly different at $p < 0.05$, p-value: Probability value and CV (%): Coefficient of variance

from seeds of V1 (23), respectively with significant differences. Overall, the interaction between seed origin and temperature levels suggests that the seed collected from V3 demonstrated a high germination potential when sown under 12 (31.3), 10 (27) and 8 (23) temperature levels followed by the seeds obtained at V2 when sown at 12 $^{\circ}\text{C}$ (28), 10 $^{\circ}\text{C}$ (23) and 8 $^{\circ}\text{C}$ (22). The seed collected in V1 12 $^{\circ}\text{C}$ (23), 10 $^{\circ}\text{C}$ (21.3) and 8 $^{\circ}\text{C}$ (21.3) performed lowest across the temperature levels throughout the experiment.

Interactive effect between seed origin and temperature on number of days to median (50%) seed germination of *Cannabis sativa* ecotypes: The results showed a highly significant difference ($p < 0.02$) in the interactions between seed origin and temperature levels on the number of days to

median (50%) seed germination (Table 2). The results showed that 50% seed emergence was recorded on the seventh DAS on seeds collected in V3 when subjected at 12 $^{\circ}\text{C}$ temperature level, closely followed by seeds collected from V2 when subjected at 10 $^{\circ}\text{C}$ on the eighth DAS and were significantly different to each other. Furthermore, the seeds obtained in V2 achieved median germination on the ninth DAS when subjected at 12 $^{\circ}\text{C}$ whilst seeds from V1 took a significantly longer period to reach the median (50%) germination irrespective of the treatment. The interaction between seed origin and temperature levels on the number of days to 50% seed germination suggested that the seed collected from V3 exhibited rapid germination potential when exposed to a 12 $^{\circ}\text{C}$ temperature level as compared to others.

Temperature effect on the seed final germination percentage (FGP%) of *Cannabis sativa* ecotypes: The results show that the final germination percentage (FGP%) was highly influenced significantly ($p < 0.001$) by the temperature regimes to which the cannabis seeds were subjected (Fig. 3). The cannabis seeds when exposed to 12 $^{\circ}\text{C}$ temperature level exhibited the highest FGP followed by the seeds exposed at 10 $^{\circ}\text{C}$ throughout the experiment, except on the twelfth DAS only when the seeds exposed at 8 $^{\circ}\text{C}$ (60.3%) recorded high FGP compared to those at 10 $^{\circ}\text{C}$ (56%). The trend continued until the fourteenth DAS when the experiment was terminated whereby the highest FGP was recorded when the seeds were subjected at 12 $^{\circ}\text{C}$ (78.4%) followed by seeds sown at 10 $^{\circ}\text{C}$ (67.9%) and the lowest germination (%) was obtained when the seeds were exposed to 8 (63.5%), respectively and were all significantly different to each other.

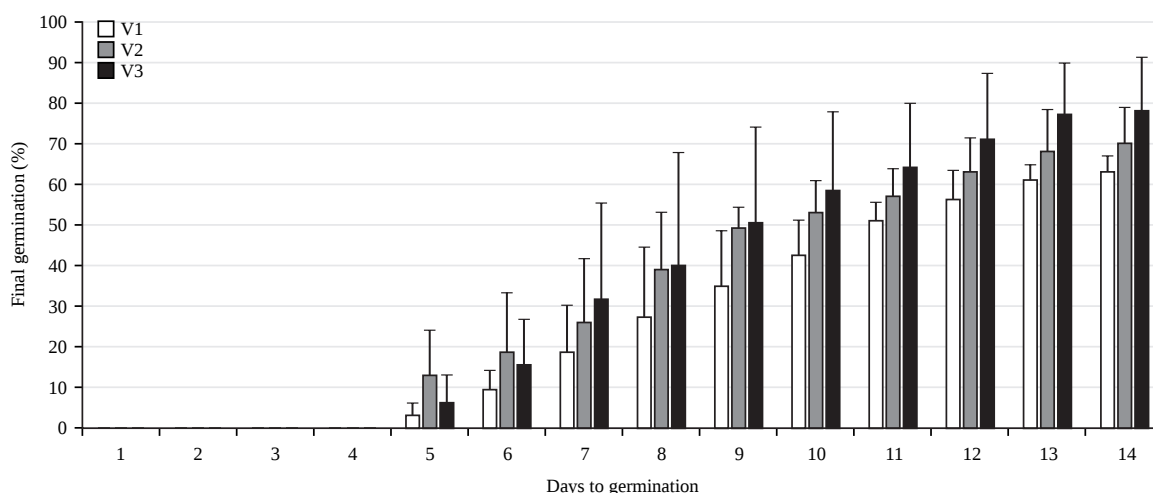


Fig. 4: Effect of seed origin on the final germination percentage (FGP %) of *Cannabis sativa* seeds collected in Lusikisiki
Bars bearing the same height indicate no significant differences ($p = 0.05$)

Table 3: Interactive effect of seed origin on final germination percentage (FGP%) of *Cannabis sativa* ecotypes

Treatment	Days to final germination percentage (FGP %)										
Seed origin $\times$ Temp ($^{\circ}\text{C}$)	Day 1-4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
V1 $\times$ 8	0	1.9 ^c	3.8 ^e	3.8 ^e	7.6 ^f	21 ^e	39 ^{ed}	48.6 ^{cc}	59.1 ^{cd}	60 ^c	61 ^c
V2 $\times$ 8	0	3.8 ^c	4.7 ^{de}	9.5 ^d	21 ^{de}	44.8 ^b	46 ^{ced}	50.5 ^c	58 ^{cd}	59 ^c	64 ^c
V3 $\times$ 8	0	3.8 ^c	7.6 ^{dce}	8.6 ^{dd}	14.3 ^{fe}	30.5 ^{ed}	47 ^{ced}	54.3 ^c	63.8 ^{cb}	66 ^c	66 ^c
V1 $\times$ 10	0	1.9 ^c	11.4 ^{dc}	27.6 ^c	27.6 ^{dc}	32.4 ^{cd}	36.2 ^e	48.6 ^{cc}	48.6 ^d	60 ^c	61 ^c
V2 $\times$ 10	0	8.6 ^{cb}	14.3 ^c	24.8 ^{cc}	48.5 ^b	50.5 ^b	53.3 ^{cb}	56.2 ^{cb}	59 ^{cd}	66 ^c	66 ^c
V3 $\times$ 10	0	3.8 ^{cb}	7.6 ^{dce}	26.7 ^c	31.4 ^{cc}	42.9	48.6 ^{ced}	56.2 ^{cb}	59 ^{cd}	77.1 ^{bb}	77.1 ^{bb}
V1 $\times$ 12	0	5.7 ^{cb}	13.3 ^{cc}	24.8 ^{cc}	46.7 ^{bb}	51.4 ^b	52.4 ^{cb}	54.3 ^c	61 ^{cb}	62 ^c	66 ^c
V2 $\times$ 12	0	26.7 ^a	37.1 ^a	43.8 ^b	47.6 ^{bb}	52.4 ^{bb}	59 ^b	63.8 ^b	71.4 ^b	78.1 ^b	80 ^b
V3 $\times$ 12	0	11.4 ^b	28.6 ^b	60 ^a	74.3 ^a	78.1 ^a	80 ^a	82 ^a	88.6 ^a	88.6 ^a	89 ^a
Mean	-	7.5	14.6	25.5	35.4	44.9	51.2	57.1	63.2	68	70
CV (%)	-	57	26	18	15.3	13.5	13	7.8	9.4	8	6.7
p-value	-	0.01	0.001	0.002	0.002	0.001	0.04	0.005	0.03	0.07	0.04

Values in a column followed by the different letters are significantly different at $p \leq 0.05$, p-value: Probability value and CV (%): Coefficient of variance

Effect of seed origin on seed final germination percentage (FGP%) of *Cannabis sativa* ecotypes:

The results of the study revealed that the seed origin had significantly ($p < 0.001$) influenced the final germination percentage (FGP %) of *Cannabis sativa* seeds (Fig. 4). The first seed protrusion was recorded on five DAS in all the treatments with the highest FGP obtained on seeds collected at V2 (13.01%) followed by V3 (6.3%) and the lowest was recorded in V1 (3.2%). A similar trend was observed through six DAS. However, on the seven DAS a significant increase in FGP was observed on seeds collected at V3 (31.7%) being the highest closely followed by V2 (26%) while the lowest was recorded at V1 (18.7%) and all significantly different to each other. A similar trend continued until the fourteenth DAS when the experiment was terminated, whereby the seeds collected at V3 (78.4%) recorded the highest FGP followed by V2 (67.8%) and the

lowest was attained in seeds collected from V1 (63%) and were all significantly different to each other.

Interactive effect of seed origin and temperature levels on the final germination percentage (FGP%) of *Cannabis sativa* seeds:

A significant ($p < 0.05$) interaction between seed origin and temperature levels has been observed in the final germination percentage of *Cannabis* ecotype seeds (Table 3). Regarding seeds exposed at 8°C temperature level: The seeds collected from V2 recorded the highest FGP from the seventh-ninth DAS followed by seeds collected at V3 and the V1 collection was the poor performer. However, a significant rise was observed on the tenth DAS when the seeds collected at V3 (47%) recorded the highest FGP followed closely by V2 (46%) seeds with no significant difference. A similar trend continued throughout the experiment until fourteenth

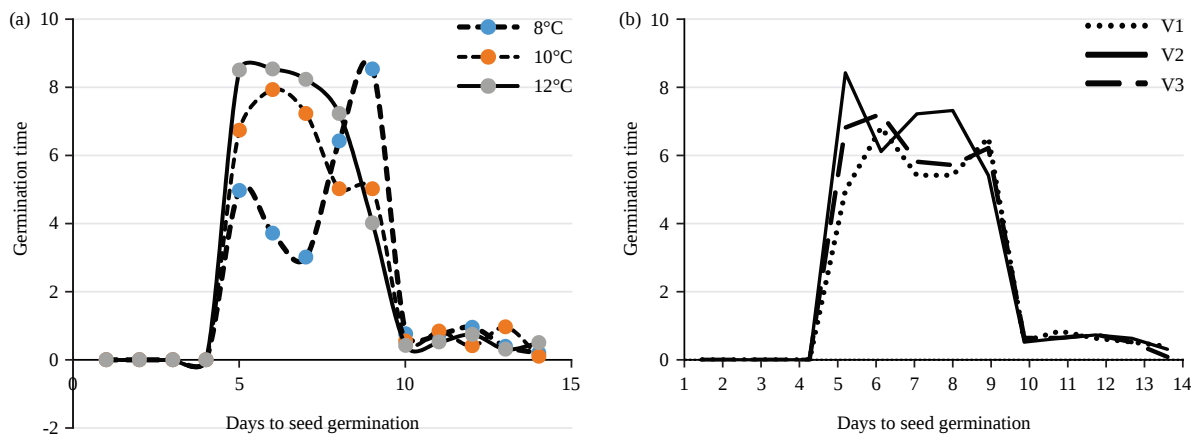


Fig. 5(a-b): (a) Effect of temperature on seed germination with reference to coefficient of velocity of germination of *Cannabis* ecotypes (CVG) and (b) Effect of seed origin on seed germination with reference to coefficient of velocity of germination of *Cannabis sativa* ecotypes seeds

Blue colour on dot = 8°C, Orange colour on dot = 10°C and grey colour on line = 12°C

DAS when seeds collected at V3 (66%) recorded the highest FGP closely followed by V2 (64%) and the lowest was at V1 (61%). A similar trend was observed when the seeds were exposed at 10°C temperature level whereby on the fourteenth DAS when the experiment terminated the seeds collected V3 (77.1%) exhibited the highest FGP followed by V2 (66%) and the lowest was obtained at V1 (61%) collection.

A significantly higher performance was observed on the seventh DAS when seeds collected at V3 (60%) demonstrated a higher FGP when subjected to 12 followed by V2 (43.8%) and the seeds collected at V1 (24.8%) performed poorly. A similar trend was observed throughout the experiment with the highest FGP attained on seeds collected from V3 (89%), followed by seeds sourced at V2 (80%) and the lowest FGP recorded on seeds obtained at V1 (66%) and were all significantly different to each other. Overall, the interaction between seed origin and temperature levels on the FGP suggested that the seed collected from V3 exhibited the highest germination potential across the experiment irrespective of treatment and has further demonstrated a rapid germination potential when exposed to a 12°C temperature level as compared to others.

Effect of temperature on the coefficient of velocity of germination (CVG) of the seeds of *Cannabis sativa* ecotypes: The results showed a non-significant temperature effect throughout the experimental period except for the fifth, sixth, seventh and twelfth DAS which were significantly different ($p < 0.05$) (Fig. 5a). The mean coefficient of velocity of germination increased with the increasing temperature and

decreased with the increasing germination period (days). The mean CVG was significantly ($p < 0.001$) higher on the fifth until the seventh DAS when the seeds were exposed to 12°C (8.5), followed by 10°C (6.7) and the lower CVG was recorded when the seeds were exposed to 8°C (3.7). On the eighth DAS, a gradual decline in CVG was observed on seeds that were exposed at 12 and 10°C temperature levels whilst an increasing CVG was observed on seeds that were subjected to 8°C. Although insignificantly rapid germination was observed on the nine (8.5) to twelfth (0.75) DAS when the seeds were exposed at 8°C. Similarly, the seed origin did not significantly influence the CVG of the cannabis seeds ecotypes except for the five DAS which were significantly different ($p < 0.002$) (Fig. 5b). The V2 (8.4) ecotype recorded the highest mean CVG followed by V3 (6.8) and V3 (4.95) recorded the least and were all significantly different from each other.

Interactive effect of seed origin and temperature levels on coefficient of velocity of germination (CVG) of *Cannabis sativa* ecotypes seeds: The results presented revealed that the interaction between the seed origin and the temperature did not significantly influence the CVG of the cannabis ecotype seeds during the experimentation period except for the seventh, eighth and twelfth DAS (Table 4). The mean coefficient velocity of germination increased with the increasing temperature levels. When the seeds were exposed to 8°C temperature level the seeds collected from V2 (8.1) recorded the highest mean CVG followed by V3 (6.9) and the lowest was attained at V1 on both the seventh and eighth DAS, respectively. A similar trend was observed when the

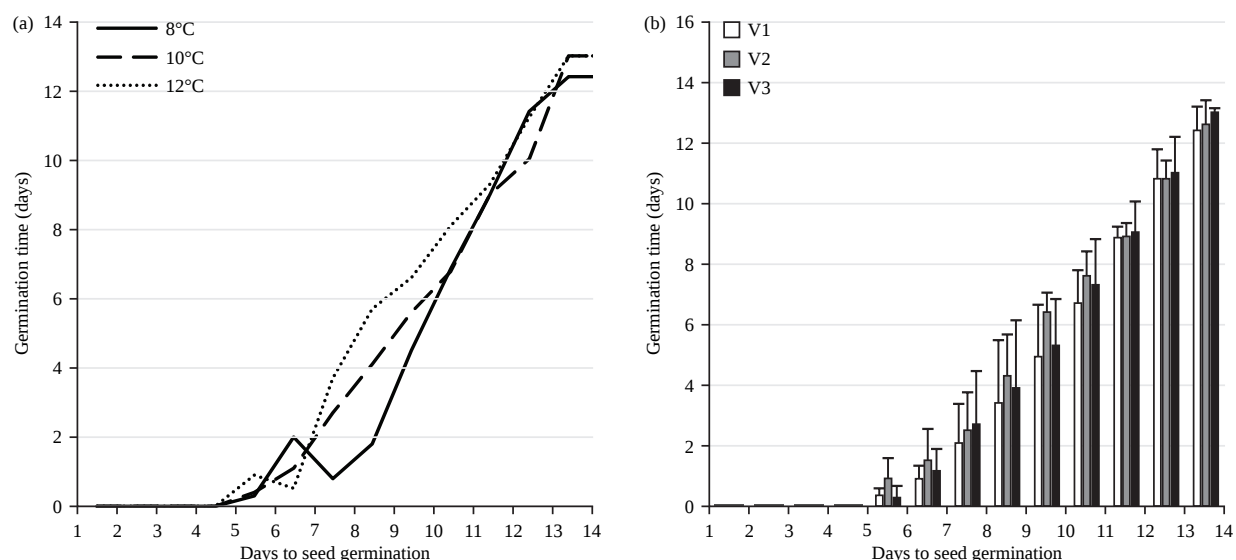


Fig. 6(a-b): (a) Effect of temperature on mean germination time (MGT) of *Cannabis sativa* ecotypes seeds and (b) Effect of seed origin on the mean germination time of *Cannabis sativa* ecotypes seeds collected in Lusikisiki
Bars bearing the same height indicate no significant differences ($p = 0.05$)

Table 4: Interactive effect of seed origin and temperature levels on coefficient of velocity of germination (CVG) of *Cannabis sativa* ecotype

Treatment	Days to coefficient of velocity of germination										
Seed origin $\times$ Temp ($^{\circ}\text{C}$)	Day 1-4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
V1 $\times$ 8	0	2.7 ^d	4.1 ^{cc}	0 ^c	4.5 ^{ba}	8.2 ^{ba}	1 ^a	1 ^a	0.96 ^a	0.29 ^{bc}	0 ^b
V2 $\times$ 8	0	7.1 ^{abc}	2.1 ^c	6.9 ^a	8.1 ^a	9 ^a	0.3 ^c	0.6 ^b	0.94 ^a	0.29 ^{bc}	0.61 ^{ba}
V3 $\times$ 8	0	5.1 ^{bdc}	4.9 ^{bc}	2 ^{bc}	6.5 ^{ba}	8.5 ^a	1 ^a	0.7 ^b	0.96 ^a	0.58 ^{bac}	0 ^b
V1 $\times$ 10	0	4.4 ^{dc}	8.5 ^a	7.7 ^a	2.6 ^b	6.3 ^{ba}	0.4 ^c	1 ^a	0.96 ^a	0.96 ^a	0.29 ^{ba}
V2 $\times$ 10	0	8.6 ^{ba}	7.4 ^{ba}	7.8 ^a	9.1 ^a	2.3 ^b	0.6 ^b	0.6 ^b	0.61 ^b	0.94 ^a	0 ^b
V3 $\times$ 10	0	7.1 ^{abc}	7.9 ^{aa}	6.1 ^{ba}	2.9 ^b	5.8 ^{ba}	0.7 ^b	1 ^a	0.61 ^b	0.98 ^a	0 ^b
V1 $\times$ 12	0	7.7 ^{abc}	7.8 ^{aa}	8.5 ^a	9 ^a	4.9 ^{ba}	0.3 ^c	0.6 ^c	0.94 ^a	0.29 ^{bc}	0.89 ^a
V2 $\times$ 12	0	9.5 ^a	8.8 ^a	6.8 ^a	4.8 ^{ba}	4.9 ^{ba}	0.7 ^b	0.6 ^b	0.65 ^b	0.64 ^{ba}	0.31 ^{ba}
V3 $\times$ 12	0	8.2 ^{abc}	8.91 ^a	9.4 ^a	7.8 ^{aa}	4.3 ^{ba}	0.3 ^e	0.3 ^e	0.64 ^b	0 ^c	0.29 ^{ba}
Mean	-	6.7	6.7	6.13	6.1	6	0.57	0.7	0.7	0.55	0.27
CV (%)	-	33.3	23.4	38.5	44.5	56.1	84	62	35	63	138.8
p-value	-	0.81	0.41	0.04	0.03	0.71	0.33	0.76	0.04	0.27	0.13

Values in a column followed by the different letter are significantly different at $p \leq 0.05$, p-value: Probability value and CV (%): Coefficient of variance

seeds were subjected at 10°C with V2 recording the highest mean CVG. However, when the seeds were subjected at 12°C the high mean CVG was attained on the seeds that were collected in V3 (9.4) closely followed by V1 (8.5) and were significantly the same. Moreover, on the twelfth DAS when the seeds collected from V1 recorded a high mean CVG irrespective of the temperature level.

Effect of temperature and the seed origin on mean germination time (MGT) of the seeds of *Cannabis sativa* ecotypes: The results revealed that the temperature levels had significantly influenced the mean germination time (MGT) of the *Cannabis sativa* ecotype seeds and they increased with the increase in temperature and the period of germination (days) (Fig. 6a). The mean MGT was higher when the seeds

were exposed at 12°C and was lower at 8°C and this trend was observed until the tenth DAS. On the eleventh DAS, the MGT reached equilibrium with the steady mean (9) across the temperature levels and a similar trend was observed between the 12 and 10°C until the fourteenth DAS when the experiment was terminated. A highly significant effect ($p < 0.00$) was observed on the MGT as influenced by the seed origin from the 5th day (the first day of germination) until the ninth DAS whereas the insignificant difference was observed from the ten to eleven DAS (Fig. 6b). The seeds collected at V2 demonstrated the significantly high MGT followed by V3 collection and the least was attained at V1. However, on the eleven to the fourteen DAS the seeds collected in V3 obtained the high MGT followed by V2 and were insignificantly different from each other.

Table 5: Interactive effect of seed origin and temperature levels on mean germination time (MGT)

Treatment	Days to mean germination time										
Seed origin × Temp (°C)	Day 1-4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
V1 × 8	0	0.2 ^c	0.4 ^e	0.4 ^e	1.0 ^e	3.1 ^e	6.4 ^{cd}	8.8 ^{cc}	11.6 ^{ba}	12.2 ^{ba}	12.2 ^{ba}
V2 × 8	0	0.2 ^c	0.5 ^e	1.04 ^{ee}	2.6 ^{dc}	6.3 ^{ba}	7.2 ^{bc}	8.7 ^{cc}	10.9 ^{ba}	11.9 ^b	11.9 ^b
V3 × 8	0	0.4 ^{cb}	0.7 ^{de}	0.9 ^e	1.7 ^{de}	4.1 ^d	7.1 ^{bc}	9.1 ^{cb}	11.6 ^{ba}	13 ^a	13 ^a
V1 × 10	0	0.2 ^c	1.1 ^{dc}	3.2 ^{cb}	3.5 ^{cc}	4.8 ^{dd}	5.9 ^d	8.8 ^c	9.6 ^d	12.8 ^{ba}	12.8 ^{ba}
V2 × 10	0	0.7 ^b	1.3 ^{cc}	2.6 ^{cd}	5.6 ^{ba}	6.9 ^a	8.2 ^{ba}	9.4 ^b	10.8 ^{bac}	13 ^a	13 ^a
V3 × 10	0	0.3 ^{cb}	0.7 ^{de}	2.4 ^d	3.3 ^{cc}	5 ^{dc}	6.2 ^{cd}	8 ^d	9.8 ^{dc}	13 ^a	13 ^a
V1 × 12	0	0.4 ^{cb}	1.2 ^c	2.6 ^d	5.7 ^{ba}	6.9 ^a	8 ^{ba}	9.1 ^{cb}	11.1 ^{ba}	12.2 ^{ba}	12.2 ^{ba}
V2 × 12	0	1.7 ^a	2.8 ^a	3.8 ^b	4.8 ^b	5.9 ^{bc}	7.4 ^{bc}	8.8 ^c	10.7 ^{bc}	12.7 ^{ba}	12.7 ^{ba}
V3 × 12	0	0.6 ^{cb}	1.9 ^b	4.7 ^a	6.6 ^a	6.9 ^a	9 ^a	10.1 ^a	11.9 ^a	12.9 ^{ba}	12.9 ^{ba}
Mean	-	0.51	1.2	2.4	3.9	5.5	7.2	8.9	10.9	12.6	12.6
CV (%)	-	54	22.4	17.5	14.4	9.7	9.1	3.7	5.9	4.2	4.2
p-value	-	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.52	0.52

Values in a column followed by the different letter are significantly different at $p \leq 0.05$, p-value: Probability value and CV (%): Coefficient of variance

Table 6: Effect of seed origin on seedling development of *Cannabis sativa* ecotypes

Treatment	Means	
Seed origin	Radicle length (cm)	Hypocotyl length (cm)
V1	1.8 ^b	2 ^a
V2	1.9 ^b	2.4 ^a
V3	2.8 ^a	2.5 ^a
Mean	2.2	2.3
CV (%)	17.4	23.3
p-value	0.00	0.02

Values in a column followed by the different letter are significantly different at $p \leq 0.05$, p-value: Probability value and CV (%): Coefficient of variance

Interactive effect of seed origin and temperature levels on mean germination time (MGT) of *Cannabis sativa* ecotypes seeds:

The results of this study revealed a highly significant ($p < 0.00$) interaction between the seed origin and the temperature levels on the mean germination time (MGT) of the cannabis ecotypes seeds during the experimentation period except for the thirteen and fourteen DAS (Table 5). The MGT increased with the increasing germination period and temperature levels. Thus, when the seeds were exposed at 8°C the seeds collected in V3 recorded the highest MGT (0.4) and the lowest was attained at V1 (0.2) on the fifth to sixth DAS, respectively. From the seventh until the tenth DAS, the seeds collected at V2 recorded the highest MGT (7.2) closely followed by V3 (7.1) and were significantly the same. On the eleventh DAS throughout the experiment, the seeds obtained at V3 recorded the high MGT compared to others. With regards to seeds exposed at 10°C, the seeds obtained in V2 recorded the highest MGT followed by V3 throughout the experiment whilst there was a steadiness between V2 and V3 on the very last days of the experiment. When the seeds were subjected at 12°C the collection from V3 recorded the highest MGT followed by V1 throughout the experiment and did not differ significantly from each other.

Effect of seed origin on seedling development of *Cannabis sativa* ecotypes:

Results revealed that radicle growth was

significantly ($p < 0.00$) influenced by the seed origin of *Cannabis sativa* ecotypes (Table 6). The longest radicle was obtained from seeds collected in V3 (2.8 cm) followed by seeds obtained at V2 (1.9 cm) and were significantly different from each other whilst the shortest radicle was attained from V1 (1.8 cm) and were significantly the same with that of V2. Similarly, the influence of seed origin had a significant ($p < 0.02$) difference in hypocotyl growth (Table 6). The longest hypocotyl was recorded on seeds obtained in V3 (2.5 cm) closely followed by V2 (2.4 cm) and the shortest was obtained on seeds collected in V1 (2 cm) and were all significantly the same.

Effect of different temperature levels on seedling development of *Cannabis sativa* ecotypes:

The results indicated that the temperature levels had influenced significantly ($p < 0.00$) the radicle growth of *Cannabis sativa* ecotypes (Table 7). The longest radicle was recorded in seeds sown at 12°C (3 cm) and was significantly different from all others, followed by 10°C (2.1 cm) and the shortest was attained at 8°C (1.6 cm) and were significantly the same. The temperature levels had significantly ($p < 0.00$) influenced the hypocotyl growth (Table 7). The longest hypocotyl was attained in seeds sown at 12°C (3.2 cm) and was significantly different from others, followed by 10°C (2.1 cm) and 8°C (1.7 cm) which were insignificantly different from each other.

Table 7: Effect of temperature on seedling development of *Cannabis sativa* ecotypes

Treatment	Means	
Temperature (°C)	Radicle length (cm)	Hypocotyl length (cm)
8	1.6 ^b	1.7 ^b
10	2.1 ^b	2 ^b
12	3 ^a	3.2 ^a
Mean	2.2	2.3
CV (%)	17.4	23.3
p-value	0.00	0.00

Values in a column followed by the different letter are significantly different at $p \leq 0.05$, p-value: Probability value and CV (%): Coefficient of variance

Table 8: Effect of interaction between seed origin and temperature levels on seedling development of *Cannabis sativa* ecotypes

Treatment	Means	
Seed origin × Temp (°C)	Radicle length (cm)	Hypocotyl length (cm)
V1 × 8	1.6 ^{cd}	1.5 ^d
V2 × 8	1.5 ^d	1.9 ^{dc}
V3 × 8	1.6 ^{cd}	1.7 ^{dc}
V1 × 10	1.7 ^{cd}	1.9 ^{dc}
V2 × 10	2.2 ^{cb}	2 ^{dc}
V3 × 10	2.5 ^b	2.1 ^{dc}
V1 × 12	2 ^{cbd}	2.6 ^{bc}
V2 × 12	2.3 ^{cb}	3.2 ^{ba}
V3 × 12	4.3 ^a	3.6 ^a
Mean	2.2	2.3
CV (%)	17.4	23.3
p-value	0.00	0.63

Values in a column followed by the different letter are significantly different at $p \leq 0.05$, p-value: Probability value and CV (%): Coefficient of variance

Effect of interaction between seed origin and temperature levels on seedling development of *Cannabis sativa* ecotypes:

The results of the interaction between seed origin and temperature levels on radicle and hypocotyl growth were presented in Table 8. The results revealed that the radicle growth was affected significantly ($p < 0.00$) by the interaction between seed origin and temperature levels. The longest radicle was recorded in V3 seeds when sown at 12°C (4.3 cm) and was significantly different from all others followed by V3 at 10°C (2.6 cm) and the shortest radicles were obtained in V2 (1.5 cm) followed by V1 (1.6 cm) and V3 (1.6 cm) when sown under 8 temperature levels, respectively with no significant difference. The interaction between seed origin and temperature levels did not significantly affect ($p < 0.63$) the growth of hypocotyl (Table 8). However, the longest hypocotyl was obtained in V3 (3.6 cm) seeds closely followed by V2 (3.2 cm) when sown at 12°C simultaneous with a slightly significant difference and the shortest was recorded on V1 (1.5 cm) when subjected to 8°C temperature level and was significantly different to V2 and V3 at 12°C.

DISCUSSION

The study revealed that the first seed sprout occurred on the first five days of the experiment irrespective of treatment with the highest number of seeds sprouted obtained at 12°C.

Kumar *et al.*³² established that at various low temperature levels, the first seed sprout of the industrial hemp occurred on the first 2 days of the experiment in all the temperature regimes. The maximum seed sprout was obtained on the thirteenth DAS in all treatments and was steady throughout until the experiment was terminated. The fibre hemp seeds sprouted when induced to different temperature regimes and the highest germination was recorded at high temperature levels^{22,23}. Subsequently, industrial hemp requires 7°C soil and 12°C air-temperatures for seed germination²³. Furthermore, the results of this study were at per with the findings of Kumar *et al.*³² who reported the maximum number of seeds sprouted on the second last day of the experiment in all treatments recorded at the high temperature level.

The study revealed that the seed origin influenced the number of seeds germinated. The first seed protrusions were recorded 5 days after seed sowing (DAS) in all the treatments with the highest mean obtained on seeds collected at V2 while V1 performed poorly. However, the maximum number seeds germinated of was recorded on the thirteenth and fourteenth DAS in all treatments when the germination reached steadiness with the highest seed germination recorded in V3 followed by V2 and the lowest was attained in seeds collected from V1. This different germination behaviour of *Cannabis sativa* seeds might be attributed to the ecological heterogeneity of the collection sites including altitude and

topography or slope orientation. Thus, V1 is situated at the lowest altitude level enclosed at the foot of two cliff mountainous valleys while V3 is located above the valleys with slopes facing North-East. Seeds from higher altitudes had higher germination rates compared to seeds in low-lying collection sites³³. The environmental heterogeneity influence of seed origin was observed on the germination performance of the *Robinia pseudoacacia* collected in Romanian District³⁴. Furthermore, the variability in seed germination responses from different localities is well documented in numerous studies particularly conducted in the Fabaceae family species^{34,35}.

The interactive effect between the seed origin and temperature suggested that the seed collected in V3 demonstrated a high germination potential irrespective of temperature level. However, the highest count was attained when the seeds were exposed at 12°C. This is attributed to the fact that plants growing in geographical areas above the escarpment are susceptible and adaptable to a wide range of environmental conditions. The germination number of *Syrian juniper* seeds differed considerably among altitudes, such that seeds from high-altitude populations germinated more than those from low-altitude populations when the cold stratification was applied^{36,37}. Knowledge of reproductive biology, particularly germination characteristics, is fundamental to understanding how species cope with ecological variation as well as for the estimation of timing of seed emergence³⁸.

Subsequently, the interactive effect of seed origin and temperature on the number of days to median (T50) seed germination suggested that the seeds obtained at V3 exhibited rapid germination potential when exposed to 12°C achieving the median germination at seven followed by V2 at 10°C on the eighth DAS. Similar findings were reported by Teixeira *et al.*³⁹ who stated that six and nine is the number of days to reach T50 for the clover cultivars sourced from temperate regions in Italy. The number of days to reach T50 decreased from 12 to 5.7 days as temperature increased from 2.5 to 17.5°C for all four cultivars⁴⁰.

Temperature plays an essential role in regulating seed germination and seedling production⁴¹. The cannabis seeds exhibited the highest FGP when exposed to 12°C (78.4%) followed by 10°C (67.9%) and the lowest was obtained when the seeds were exposed to 8°C (63.5%), respectively. The maximum germination percentage (90%) was recorded at the optimum temperature of 12.5°C³⁹. Seed germination percentage increased from the minimum up to the optimum temperature⁴². The lowest germination of 74% when the seeds were induced under the 10°C was reported by Muhl *et al.*⁴³.

In contrast, industrial cannabis seeds did not germinate when exposed to 15°C and the FGP was reduced to approximately 15% when subjected to 30°C^{18,44}. The current study revealed that the seed origin had significantly influenced the FGP of *Cannabis sativa* seeds. The highest FGP was attained on seeds collected at V3 (78.4%) followed by V2 (67.8%) and V1 (63%) performed the least. Kumar *et al.*³² reported maximum germination of *Cannabis sativa* seeds collected in Kausani Village compared to others. The seeds collected in areas of higher geographic elevation have exhibited a good germination potential compared to those of low-lying areas^{45,46}. Interaction between seed origin and temperature on the FGP suggested that the seed collected from V3 exhibited the highest germination potential irrespective of the treatment and has further demonstrated a rapid germination potential when exposed to 12°C temperature level as compared to others. The geographic elevation and slope orientation played a significant role in the influence of environmental variables that are critical for seed germination and development of Cannabis⁴⁷. Similar findings were reported earlier by Mariko *et al.*⁴⁸ that a significantly higher percentage and speed of germination was observed on the upland populations than the lowland populations when sown under the lowest temperature regime of 10 and 15°C. Moreover, the environmental effect on phenology is evident with larger differences in altitude due to variable photoperiod sensitivity among genotypes^{49,50}. Thus, the higher altitudes may compensate for some of the decreases in thermal sensation caused by a cold environment⁵¹.

The seed's germination rapidity is indicated through the coefficient of velocity of germination (CVG)⁵². This study showed that the mean CVG increased with the increasing temperature and decreased with the increasing germination period (days) except for seeds induced at 8°C who recorded a high CVG on the ninth day of the experiment. According to Botey *et al.*⁵³ CVG increased with the increasing number of germinated seeds and decrease in time required for germination. From the present study, it has been established that the CVG was influenced by temperature levels, suggesting that when the seed is exposed to increasing temperatures, it requires less time to germinate and at a faster germination rate. Thus, the CVG was significantly higher on the fifth until the seventh DAS when the seeds were exposed at 12°C followed by 10°C. Martins *et al.*⁵⁴ observed similar findings in African eggplant where seeds induced at increased temperature levels had a faster germinability compared to low temperature exposure. However, Sleimi *et al.*⁵⁵ contradicted the finding of this study when they noted delayed germination with the rise in temperature from 15-30°C in

chickpeas. In addition, it has been established from this study that the CGV is affected by the seed origin or locality where the seeds were collected. Thus, the V2 ecotype recorded the highest mean CVG followed by V3 and V1 recorded the least. This might be attributed to geographic orientation of the V2 which is the south facing slope as well as the low-lying area in which the village is situated. Elevational patterns occurred in seed germination led to a shorter germination time on seeds collected at higher elevation⁵⁶. The MGT is a measurement of the average length of time required for maximum germination of a seed lot⁵⁷. It is an accurate measure of the time taken for a seed to germinate⁵⁸. In the present study, a highly significant ($p < 0.00$) interaction between the seed origin and the temperature levels on the mean germination time (MGT) of the cannabis ecotypes seeds was observed during the experimentation period, except for the thirteenth and fourteenth DAS. The MGT increased with the increasing germination period and temperature level. Seeds collected from V3 demonstrated high germination rapidity irrespective of the temperature treatment, closely followed by V2 inconsistently and the V1 seeds had the longest germinability. This may be attributed to the fact that V3 and V2 are situated in the high escarpment, with V2 located in the south facing slope making the plants growing in these villages prone to the frequent fluctuating temperatures compared to that of V1 which is located at the low warm elevations. According to Wu *et al.*⁵⁹ seeds collected from high altitudes are more sensitive to temperature and are likely to benefit from the higher incubation temperature with increasing germination percentage and shorter germination timing. The results of this study were confirmed by the findings of Bankar *et al.*⁵² who reported that the interaction between the genotype and temperature resulted in a higher MGT at 15°C. Furthermore, Joshi and Dhar⁶⁰ reported that reducing the duration of exposure to chilling temperatures has improved seed germination and increased MGT.

Seed origin significantly ($p < 0.01$) influenced the radicle growth of *Cannabis sativa* ecotypes with the longest radicle obtained from seeds collected in V3, whilst the shortest radicle was attained from V1. A similar trend was observed with regard to hypocotyl. The maternal environment during the seed production period influenced germination responses, seed traits and radicle growth⁶¹. Earlier on, the plant physiologist, Tranquillini⁶² reported that growth conditions for plants in mountainous areas are increasingly less favourable with increasing altitude, as a result, plants adapted to such conditions can survive decreases in temperature levels. According to Cooper⁶³ plants develop faster at warm low-lying altitudes; their growth rate is dependent on soil and air temperature. This justified the poor performance exhibited by

seeds collected from V1 as it is situated in low-lying altitude with mother plants adapted to warm conditions. Considering the effect of different temperature levels on seedling development of *Cannabis sativa* ecotypes, a significant difference was recorded on both the radicle and hypocotyl with the longest recorded at 12°C. The findings agreed with Roman *et al.*³⁴ who reported that the exposure of *Robinia pseudoacacia* to heat/low temperature positively influenced the growth of the root and shoot. Seeds subjected to high-temperature regimes demonstrated the longest radicle and hypocotyl compared to those at the lowest temperatures²³.

A significant interaction was observed between seed origin and temperature on radicle growth. The radicle growth was highly ($p < 0.00$) affected by the interaction between seed origin and temperature levels with the longest radicle recorded in V3 seeds irrespective of temperature level followed by V2. Although insignificant, a similar trend was observed regarding hypocotyl length. Environmental and growing conditions during the seed development and morphological maturation affect the emergence of radicle, hypocotyl and generally the plant seed quality^{20,64,65}. These findings were confirmed by Varga *et al.*²³ who reported that at the lowest temperature, 10°C the seedlings were the shortest, while the highest temperature (20°C) stimulated seedlings elongation. While in contradiction with Assirelli *et al.*²² who found that the 7°C minimum temperature for normal germination of *Cannabis sativa* seeds.

Considering the findings of this study it may be recommended that the medicinal cannabis seedlings may be produced immensely when the minimum temperature is 12°C to ensure rapid seed germination. Moreover, seeds produced from mother plants in village 3 could be a preferred choice for cannabis seedling production as they demonstrated adaptation plasticity across low-temperature regimes.

CONCLUSION

In the present study, the findings suggested that *Cannabis sativa* seeds begin germinating 5 days after sowing at low temperatures, with the optimal germination rates observed at 12°C. Seeds collected from a high-altitude area showed the best overall performance, with rapid and more successful germination, as well as superior seedling development. This is evident as key germination indices, including T50, FGP, CVG and MGT, supported these findings, indicating that 12°C is optimal for cannabis seed germination and growth. It will be interesting to further explore the effects of wider varying temperature ranges and other environmental factors on different cannabis ecotypes to enhance germination strategies for broader cultivation.

SIGNIFICANCE STATEMENT

The study evaluated the germination response of *Cannabis sativa* seeds under varying minimal temperatures to improve its cultivation practices. The study finds that cannabis seeds begin germinating five DAS regardless of temperatures; however, germination rates peaked as temperature increased, with 12°C being the optimal temperature for seed germination. Furthermore, seeds of higher altitude areas also germinated optimally. These findings can serve as valuable inputs towards improving *Cannabis sativa* production. Based on the results of this study can be recommended that medicinal cannabis seedlings be produced immensely when minimum temperature is 12°C to ensure rapid seed germination and seedling development. Moreover, seed obtained in areas of high altitude could be a preferred choice for cannabis seedling production as they demonstrated adaptation plasticity across minimal temperature regimes.

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