RESEARCH ARTICLE

SEED GERMINATION BEHAVIOUR OF *CANNABIS SATIVA* L. UNDER DIFFERENT TEMPERATURE REGIMES

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A**bstract:** *Cannabis sativa* L. (Cannabaceae) is one of the earliest cultivated plant, containing many of the valuable natural components useful for health as well as livelihood. Cultivation of *Cannabis* is done by sowing its seeds in the field provided with favourable physical and chemical parameters for germination. In this study, optimum temperature and time required for germination of *Cannabis* seeds collected from Kausani, Uttarakhand have been studied at various temperatures under the controlled laboratory conditions. The percentage of germination, germination energy and seedling vigor index I and II was reported maximum at a constant temperature of '25˚C' with having 3rd-4th and 6th day as its first and final count day, respectively. Therefore, it is suggested to the researchers/cultivators to raise the nursery of *Cannabis sativa* L. seed at '25˚C' to achieve healthy and maximum seedlings of the crop.

Keywords: Hemp, THC, CBD, Germination potential, Seedling vigor

INTRODUCTION

Cannabis sativa L. (Fam. Cannabaceae), an annual herb, native to eastern Asia is cultivated worldwide having medicinal and recreational properties due to the presence of more than 100 active chemical constituents viz. Cannabidiol (CBD), Tetrahydrocannabinol (THC), Cannabinol (CBN), Tetrahydrocannabivarin (THCV), THCa, and cannabidiol terpenes, etc. (Sera et al., 2018). Being a good source of fibre, it can be used in paper, cotton, biodegradable plastics, paints and bio-fuel industries (Keller, 2013; Johnson, 2019; Fortenbery and Mick, 2015). Major cultivated areas for *Cannabis* production in the world are Europe, Canada and China (Huaran et al., 2018). One third to half of the total *Cannabis* cultivated area of the world is occupied by China (Yan, 2014; Salentijn et al., 2015). Under section 10 of The NDPS Act, 1985 in India, under section 41 of UP Excise Act 1910 (UP Excise Rule, 2018) and section of 14 of The NDPS Act for hemp cultivation rule in Uttarakhand (Uttarakhand Hemp Cultivation Rule, 2016-17) a proper licence required from state excise department for the cultivation of *Cannabis* for medicinal and scientific purposes (The NDPS Act, 1985; Official Gazette of UP, 2018). Government of India, UP state excise department and Uttarakhand state excise department permits the cultivation of only those varieties/genotypes of *Cannabis* which have less than 0.3% THC and higher content of CBD (The NDPS Act, 1985; Official Gazette of UP, 2018).

Dioecious *Cannabis* herb can be cultivated through seeds in the field. Seed germination potential is a very important aspect for the successful establishment of seedling in the field and commercial cultivation of *Cannabis* crop. Germination of *Cannabis* seed is tested under the controlled condition of the laboratory by examining the emergence and development of seedling where the condition of its essential structures (root system, shoot axis, cotyledons, terminal buds) determine its ability to grow under the favourable condition in the field (ISTA Rule, 2010). Temperature variation along with a number of days to counting affected the germination percentage of *Ocimum basilicum* (Kumar et al., 2012), *Tagetes minuta* (Kumar et al., 2008), *Cymbopogon martinii* (Kumar et al., 2010) and *Artimisia annua* (Kumar et al., 2013). Change in the temperature and light had also affected the germination of lettuce seeds (Erwin, 1991.However a very few and erratic information is present depicting the optimum temperature and photoperiod required for the seed germination of *Cannabis sativa* L. Thus, the main motive of this study was to determine the optimal temperature regimes and time required for maximum seed germination potential. The results of maximum seed germination percentage obtained in the seed germinator under controlled laboratory condition are accepted worldwide by seed trades and

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its customers as indicators of field germination (Yaklich and Kulik, 1979).

MATERIALS AND METHODS

Seed collection

The *Cannabis sativa* L. seeds were collected during October, 2019 from their naturally growing habitat of Kausani and Bageshwar regions of Uttarakhand, India. The seeds were stored in paper bag at '25°C±3°C' until the germination experimentation was initiated.

Germination potential and seedling vigor index

During December, 2019–January, 2020 experiment were conducted at constant temperatures of '15°C', '20°C', '25°C', '30°C', '35°C' and '40°C' at 16 h light/8 h dark daily regimes and alternate temperatures of '25°C/15°C' at 3 photo-regimes viz.16h light/8hr dark, 8h light/16h dark and 12h light/12h dark with 60-70% relative humidity. Seeds were placed on top of filter paper (TP) soaked with sterile distilled water in Petri dishes (15cm diameter × 3 cm deep). The experiment was arranged in a complete randomized design with six replications of 50 seeds. Germination was checked daily and numbers of normal (bearing both root and shoot) and abnormal (lacking either root or shoot or having stunted growth) seedlings recorded from the first day of counting till the day of maximum seed germination percentage (Kumar et al., 2011). Numbers of healthy seedlings were used for data analysis. Observation on germination percentage, germination energy percentage, seedling vigor index I and seedling vigor index II were recorded and calculated (Kumar et al., 2011) as follows:

$$\text{Germination percentage} = \frac{\text{Total number of seed}}{\text{Total number of seeds in all replicates}} \times 100$$

$$\text{Germination energy} = \frac{\text{¼ of maximum number of seeds germinated in a day}}{\text{Total number of seeds in all replicates}} \times 100$$

Seedling vigor index I = Germination (%) x Average seedling length (cm)

From each replication, ten normal seedlings were selected randomly at the end of the germination test, and seedling length (root + shoot length) was measured. Average seedling length (cm) was calculated.

Seedling vigor index II = Germination (%) x Average seedling dry weight (g)

The same ten seedlings were placed in a paper envelope and dried under shade for 16 h. These dried seedlings were placed in an oven at 75°C±5°C for 48 h after which they were weighed to determine average seedling dry mass (g) in each replication.

Statistical analysis

At the end of the experiment, data were subjected to an analysis of variance (ANOVA) and mean separation. The least significant difference (LSD) at 5% level was used to compare the means of different test parameters under different temperature conditions.

RESULTS AND DISCUSSION

ANOVA of table 1 revealed that temperature regimes (T), number of days to counting (D) and their interaction were highly significant for percentage of germination (G) and germination energy (GE) while table 2 revealed that temperature regimes was highly significant for percentage of germination (G), seedling vigor index I (SV-I) and seedling vigor index II (SV-II) of *Cannabis* seed. Percentage of germination (G) along with germination energy (GE) was analysed to be varying from day to day at different temperature regimes. At various temperature regimes, the emergence of the radicle was recorded during the first two days of the experiment except at '40°C' where no seed germination was observed though out the experiment. While at 35°C, radicle and plumule emerged on 2nd and 3rd day respectively showing stunted growth (devoid of cotyledon) that later died on 4th day of experiment. The appearance of both radicle as well as plumule was firstly observed on day 3rd at all studied temperature regimes [15°C', '20°C', '25°C' (16h/8h), '25/15°C' (16h/8h), '25/15°C' (8h/16h) and '25/15°C' (12h/12h)].The maximum seed germination was found on day 6th at all studied temperature regimes.

The mean percentage of seed germination and germination energy over the temperature regimes for number of days to counting varied from 37.83 and 9.46 ('15°C') to 62.38 and 15.84 ('25°C'), respectively (Table 3). Among the studied temperature regimes, '25°C' had the highest mean percentage of germination and germination energy (63.38 and 15.84) and significantly different from other temperature regimes while among the number of days to counting, day 6th has maximum mean percentage of germination and germination energy (70.36 and 17.59) and significantly different from rest days of counting. Considering these two factors simultaneously i.e. a number of days to counting and temperature regimes, '25°C' was found the best with the maximum mean percentage of germination and germination energy (87.17 and 21.79) and was followed by'30°C' (77.33 and 19.33), '25/15°C (8h/16h)' (76.67 and 19.17), '25/15°C (16h/8h)' (71.00 and 17.75), '25/15°C (12h/12h)' (70.83 and 17.71), '20°C' (60.17 and 15.04), and '15°C' (49.33 and 12.33) at day 6th of temperature regimes (Table 3). Mean of seed germination percentage (G) was

found maximum (87.17%) at constant temperature of '25°C' among the studied temperature regimes over the number of days to counting, seemed to be optimum temperature (Table 3). Similar observations have also been reported by various workers in the case of Indian basil (Kumar, 2012), sweet basil (Ramin, 2006), Holy basil (Kumar et al., 2014), Palmarosa (Verma et al., 2012) and Kalmegh (Kumar et al., 2011). Analogously, for a number of days to counting, day 6th had the highest values for the percentage of germination and germination energy (Table 3).Variation in seedling vigor index I (SV-I) and seedling vigor index II (SV-II) was also recorded with the change in temperature (Table 4). Seedling vigor I was found maximum (758.98) at '25/15^{o}C (8h/16h)' and significantly different from other temperature regimes except at '25/15°C (16h/8h)' while seedling vigor index II (12.32) at a constant temperature of '25^{o}C' was significantly different from other temperature regimes (Table 4). The finding of the present study regarding maximum seedling vigor index I at '25/15°C (8h/16h)' and reduction in its value by altering the temperature or photoperiod suggests that on changing the temperature and relative humidity, viability and vigor of seed got disturbed as reported in the case of the *Ocimum basilicum* L. (Kumar, 2012). In case of maximum value of seedling vigor II at '25°C' is defended by similar findings obtained for *Lens culinaris* (Khatun, 2009) and Indian basil (Kumar, 2012). Seedling vigor index I (451.72) and II (6.15) were reported minimum at a temperature of '20°C' and '15°C', respectively (Table 4). The lowest seedling vigor index II at '15°C' could be due to lesser dry mass of seedling and lower value of germination percentage similar to the case reported for Indian basil (Kumar, 2012). Another reason for decreased viability and vigor of seeds was because of earlier harvesting the seeds before the accomplishment of their physical maturity which increases the number of seeds with relatively low embryonic development and higher content of moisture in them as suggested in case of *Lens culinaris* (Khatun, 2009) and *Pisum sativum* (Matthew, 1973).

Table 1. Analysis of variance for percentage of germination (G) and germination energy (GE) in *Cannabis sativa* L. under different temperature regimes (T).

Sources	df	Mean Squares	
		G	GE
Replication	5	10.79761905	0.590029762
Temperature regimes (T)	6	823.297619**	51.14236111**
Error1	12	29.91220238	1.921843998
Number of days to counting (D)	3	6019.439484**	376.8027654**
T x D	18	109.228836**	6.769345238**
Error2	42	7.800595238	0.504402282

** Significant at probability level ($p \leq 0.001$)

Table 2. Analysis of variance for percentage of germination (G), seedling vigor index I (SVI) and seedling vigor index II (SVII) in *Cannabis sativa* L. under different temperature regimes (T).

Sources	df	Mean Squares		
		Germination %	SV I	SV II
Replication	5	1.715	2459.64	0.50
Temperature regimes	6	458.76**	39307.92**	10.52**
Error	12	14.01	1939.70	0.31
Total	20	103952.68	6828087.99	1939.03

** Significant at probability level ($p \leq 0.001$)

Table 3. Percentage of mean germination (G) and germination energy (GE) of *Cannabis sativa* L. at number of days to counting (D) under different temperature (T) regimes.

Temperature(T) regimes	Number of Days to counting (D)									
	Day 3		Day 4		Day 5		Day 6		Mean	
	G	GE	G	GE	G	GE	G	GE	G	GE
15°C (16h/8h)	28.67	7.17	34.00	8.50	39.33	9.83	49.33	12.33	37.83	9.46
20°C (16h/8h)	37.00	9.25	47.33	11.83	57.00	14.25	60.17	15.04	50.38	12.59
25°C (16h/8h)	40.67	10.17	53.67	13.42	72.00	18.00	87.17	21.79	63.38	15.84

30°C (16h/8h)	28.67	7.17	44.67	11.17	67.67	16.92	77.33	19.33	54.58	13.65
25/15°C (16h/8h)	22.33	5.58	34.00	8.50	46.00	11.67	71.00	17.75	43.33	10.88
25/15°C (8h/16h)	30.00	7.50	45.33	11.33	64.00	16.00	76.67	19.17	54.00	13.50
25/15°C (12h/12h)	31.33	7.83	50.33	12.58	59.67	14.92	70.83	17.71	53.04	13.26
Mean	31.24	7.81	44.19	11.05	57.95	14.51	70.36	17.59		
CD 5% for T		4.87(G)	1.23(GE)							
CD 5% for D		6.52(G)	1.62(GE)							
CD 5% for T×D		4.61(G)	1.17(GE)							

Table 4. Percentage of mean germination (G), seedling vigor index I (SVI) and seedling vigor index II (SVII) of *Cannabis sativa* L. under different temperature (T) regimes.

Temperature (T) regimes	Mean germination percentage (G)	SV I	SV II
15°C (16h/8h)	49.33	480.50	6.15
20°C (16h/8h)	60.17	451.72	9.67
25°C (16h/8h)	87.17	520.05	12.32
30°C (16h/8h)	77.33	507.78	9.74
25/15°C (16h/8h)	71.00	582.58	9.78
25/15°C (8h/16h)	76.67	758.98	10.69
25/15°C (12h/12h)	70.83	689.90	8.93
SEM	3.06	35.96	0.45
CD at 5%	6.37	74.98	0.95
CV	5.32	7.72	5.79

CONCLUSION

Percentage of germination (G) and germination energy (GE) along with seedling vigor index II for *Cannabis sativa* of Kausani accession was found maximum at a constant temperature of '25°C' with 3rd - 4th day and 6th day as its first and final count day, respectively. Thus, from the above findings it is suggested that to grow the seeds of *Cannabis* at '25°C' as its optimum temperature which will be useful for researchers/cultivators in producing seedlings for commercial cultivation.

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