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STUDIES ON EFFECT OF SPACING AND NITROGEN ON FALSE FLAX (*CAMELINA SATIVA* CV *CALENA*) UNDER CENTRAL-WESTERN HIMALAYAS OF INDIA

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ABSTRACT

False flax [*Camelina sativa* (L.) Crantz.] popularly known as Gold-of pleasure a proposed biofuel species is an oil seed crop of *Brassicacea* family introduced in India in 2009. An experiment was conducted during 2011-13 at Defence Institute of Bio-Energy Research, Field Station- Pithoragarh, Uttarakhand, India to investigate the effect of spacing and nitrogen fertilization on *C sativa* cv Calena for the large scale multiplication and production of seed for biofuel purpose. Field trials were designed in Split plot arrangement based on Randomized Complete Block Design with three replications. Main plots were spacing (RXP) including 30X10 and 30X15cm and subplots were nitrogen (Urea, 46% nitrogen) including zero, 30, 60, 90 and 120kg/ha. Results showed that different levels of spacing had significant ($P < 0.05$) effect on flowering and pod formation (days), branch/plant, plant height, pods/plant, seed as well as oil yield except oil content per centage. The addition of nitrogen fertilizer had significant effects on all the studied growth and yield parameters of *Camelina*. Statistical interpretation at $P < 0.05$ reveals, the maximum seeds/pod (12.00 No), pods/plant (152.00 No), seed yield (910.47kg/ha), oil content (36.10%) and oil yield (369.303lit/ha) were noticed for the crop with spacing of 30X15cm along with the N fertilization of 30kg/ha. This study suggests that optimum spacing as well N- fertilization increase the productivity of *Camelina* as emerging biofuel crop.

INTRODUCTION

Camelina (False flax, gold-of-pleasure, *Camelina sativa* [L.] Crtz.) is under-exploited oilseed crop of origin Mediterranean to Central Asia and belongs to *Brassicaceae* family. *Camelina* has unusual fatty acid compostition with high level alpha-linolenic acid vis–a-vis unnaturally high cholesterol (200mg/kg) and brassicacasterol content (188-133ppm) than other vegetable oils. It is short season crop 85-100 days, tolerant to frost, contains higher seed oil (320-480g/kg) and yields up to 600-1700kg/ha. The possible industrial applications of *Camelina* include environmentally safe paintings, coatings and cosmetics (Waraich et *al*., 2013). There has been recent interest in *Camelina* because of presence of high cholesterol (200mg/kg) and eicosenoic acid (15%) pose a hurdle for its approval as food oil and regarded as potential as a low-cost feed stock for biofuel production. As India is stepping up the National Biofuel Policy to meet the requirement of renewable sources of fuel and realize self-reliance in energy there is proposal of blending of 20% biofuels with petrol-diesel by 2017, *Camelina* is introduced in India from Austria in 2009 as a potential bio diesel crop that don't interferes the edible oil trade and competes for available resources (Agarwal et *al*., 2010).

Optimum plant spacing is an agronomic factor which plays a vital role in achieving the potential yield of a crop by creating optimum growing environment for growth and development that results in better yield attributes (Singh et *al*., 2002) Optimizing light interception through manipulation of plant spacing directly influences the plant physiology, growth and yield. Diepenbrock, 2000; Kumari et *al*., 2012 reports that plant spacing affects *Camelina* productivity significantly.

Nitrogen is one of the major elements for growth and development of plant. It is involved in photosynthesis, respiration and protein synthesis. It impart the dark green colour of the leaves, promotes vigorous vegetative growth and more efficient use of available inputs finally leads to higher productivity (Patel et *al*., 2013). *Brassica* is also indirectly affected by N as a result of increased stem length, higher number of flowering branches, total plant weight, seeds per pod and number and weight of pods and seeds per plant (Rathke *et al.*, 2005, Kazemeini *et al.,* 2010).*Camelina,* however, is reported to have low nutritional requirements and is also able to produce moderate yields on poorer soils than most oilseed crops. Studies have reported different N requirement for Camelina ranging from 45-90kg/ha (Wysocki *et al.,* 2013), 60-80Kg/ha (Urbanaik *et al.,* 2008) and 120-160kg/ha (Jiang *et al.,* 2013) at different locations of Moscow, USA and Canada, respectively.

Proper plant stand is the primary factor that influences the Sunflower, oilseed productivity; while the efficient use of nutrients has also been proved markedly beneficial to the crop yields (Soomro, 2015). Fathi *et al.* (2002) reported that increasing nitrogen fertilizer and plant density caused a boost in seed yield in rapeseed. The highest yield per hectare in this study resulted from 225 kg N/ha at a plant density of 90 plants per square meter. Ali *et al.,* 2013 suggested that the increasing plant density increases sunflower final achene yield but if it is combined with optimum level of nitrogen.

Assuming the present demand for biofuel, it is imperative to enhance the *Camelina* productivity through appropriate spacing and nitrogen fertilization for wider adaptability to agro climatic conditions of Central Western Himalayas of India. The present experiment was therefore attempted to study the effect of different spacing and nitrogen fertilization on growth, yield and yield components of *C. sativa*.

MATERIALS AND METHODS

The field experiment was conducted on *C. sativa* cv Calena (EC-643910), during the winter (rabi) season of 2011-2012 and 2012-13 at Defence Institute of Bio-Energy Research (DIBER) Field station Pithoragarh (Uttarakhand), situated in Central Western Himalayas of India, which extends from 29º 29′ - 30º49′N latitude to 85º05′- 81º31′E longitude and at 1700 m amsl The soil was gravel sandy - clay loam in texture, organic carbon (0.36%), N (250kg/ha), P (11kg/ha), K (115kg/ha) and soil pH varies from 6.1-7.5. Experiment was laid out in split plot design with 03 replications and comprised of spacing (RXP) as main plot treatment and nitrogen fertilization as sub plot treatment. Two different spacing 30X10 and 30X15cm, denotes as S_1 and S_2,respectively with five different nitrogen dose of 0(control),30,60,90 and 120kg/ha, denotes as N_1,N_2,N_3,N_4 and N_5, respectively was executed as treatment codes in the study. The crop was sown in mid October using seed rate of 4.5kg/ha and harvested in mid February. Nitrogen was applied through urea (46% N) in two split doses, half at field preparations and remaining half dose of N was applied after first irrigation. Two irrigations were given to the crop as life saving measure first at early seeding stage and then at pre-flowering stage of *Camelina*. No plant protection measure was taken during the studies. Days to flower and pod were determined as the number of days from date of seeding to approximately 50% flowering and pod formation, respectively. All the data were collected on plot basis and fifteen representative plants were randomly selected from each plot. Then, the plant height, the number of pods/plant, the number of seeds/pod, and 1000 seed weight were recorded. Seed yield was recorded per plot and then converted into kg/ha. The oil percentage of seeds was measured by solvent petroleum ether using Soxhlet method in laboratory (Abubakar *et al.,* 2014). The oil yield per unit area (lit/ha) was determined as the product of seed yield multiplied by oil percentage divided by the density of the *Camelina* oil (0.89g/cc). The two years data were pooled for various parameters and were subjected to analysis of variance (ANOVA) technique according to the for Split plot design using the statistical programme OPSTAT (www.hau.ernet.in/opstat.html) to draw inference of the results. Valid conclusions were drawn only on significant differences between treatment means at P=0.05 level of probability.

RESULTS AND DISCUSSION

The results of pooled analysis of the experiment conducted during 2011-13 reveals; there was significant effect of spacing and nitrogen on variable traits of *Camelina* under the study (Table 1).

Flowering duration (days)

According to results, the spacing showed significant differences in flowering at 5% probability level (Table 1).The flowering found much earlier (48.14days) in 30X15cm (Table 2) of spacing and keeps on increasing (50.86 days) towards narrower spacing of 30X10cm. Less plant competition for water, nutrients and light in low plant population plots increased vegetative growth and delayed flowering (Iqbal *et al.,* 2007). Kumari *et al.*, 2012 suggests wider spacing resulted in early flowering in *Camelina*. The effect of N level on this trait was significant at 5% level, too (Table 1). Higher N levels resulted in increasing the duration of flowering from 47.19 to 52.50 days (Table 2), revealing the fact that excess rate of nitrogen enhanced the vegetative growth of aerial parts and prolongs the period of flowering (Mehdi *et al.,* 2010). The interaction between the spacing and N does not affect this trait at 5% level (Table 3).

Pod formation (days)

It was evident from the Table 1, the pod formation was significantly affected by spacing and N fertilization and follows the similar trend as that of in flowering. Average pod formation took lesser time (58.46days) in 30X15cm of spacing (Table 2). Increase in N level significantly affected and increased the pod formation duration from 57.16 to 70.32days (Table 2). The interaction between spacing and N-levels was significant on this trait at 5% level (Table 3). The earliest pod formation (56.65days) was obtained at the density of 30X10cm with the application of 0kg N/ha (Table 3). This might be due to optimum utilization of available resources to crop at appropriate spacing that led to early onset of flowering in *Camelina* and ultimately to pod formation.

Number of branches/plant

The results showed that there were significant differences in number of branches/plant among different spacing (Table 1).The plants at 30X15cm spacing had the highest branches (14.36) compared to 8.60 in 30X10cm spacing (Table 2). The effect of N on this trait was significant at 5% level, too (Table 2). The number of branch/plant increased with the N-level so that the highest and lowest branches (14.58 and 8.00) were observed in the plants fertilized with 120kg N/ha and 0kg N/

Table 1: Analysis of variation for the studied agronomic traits of *Camelina sativa* cv calena

SourceOfvariation	df	Flowering (days)	Pod formation (days)	Branch /plant(No)	Plant height (cm)	Pod/ plant (No)	Seed/ pod (No)	1000 seed weight (gm)	Yield (kg/ ha)	Oil(%)
Replication	2	0.200	12.80	0.981	17.62	23.54	5.79	17.33	11.65	0.634
Factor A(Spacing)	1	28.488*	1128.533**	360.188*	89.86**	5504.01*	3.162 ns	16.70**	6378.31*	7.337ns
Error (a)	2	2.867	24.267	0.517	4.197	23.23	4.321	11.56	65.48	0.008
Factor B(Nitrogen)	4	66.133*	603.533**	159.538*	1298.50**	29436.55**	18.12**	0.193**	255196.3**	3.383**
Interaction A X B	4	76.67ns	747.800*	104.696*	70.03*	1546.86**	0.34ns	69.78ns	1566.35**	0.709 *
Error (b)	16	13.60	400.267	27.163	79.56	103.91	7.46	1.182	625.325	0.096

*Significant, ** highly significant and ns: Non significant at P= 0.05 level.

Table 2: Effect of spacing and nitrogen fertilization on physiology, seed yield and oil of *C. sativa* (mean of two years)

Treatments with code	Flowering (days)	Pod formation (days)	Branch/ plant(No)	Plant height (cm)	Pod/ plant(No)	Seed/ pod(No)	1000 seed weight(gm)	Seed Yield (kg/ha)	Oil(%)
Main Treatments									
30X10 cm(S1)	50.86	70.74	8.60	62.77	84.28	10.02	0.882	727.07	34.38
30X15 cm(S2)	48.14	58.46	14.36	66.24	111.37	10.67	0.929	825.20	35.57
CD (P=0.05)	2.02	5.89	1.608	2.21	5.204	NS	0.367	2.16	NS
Sem	0.309	0.899	0.131	0.37	2.69	3.15	1.81	5.42	0.117
CV	2.31	1.27	1.064	2.24	3.483	1.420	2.653	4.77	0.255
Sub Treatments									
0 N kg/ha(N1)	47.19	57.16	8.00	56.17	125.02	10.67	0.876	831.71	34.54
30 N kg/ha(N2)	48.33	59.65	9.61	59.10	134.02	11.60	0.780	877.93	35.38
60 N kg/ha (N3)	49.00	66.60	12.10	65.60	105.37	10.38	0.905	763.47	35.10
90 N kg/ha (N4)	50.49	69.36	13.03	66.57	73.50	9.67	0.946	725.37	34.99
120 N kg/ha (N5)	52.50	70.32	14.58	75.10	51.00	9.40	1.024	682.19	34.88
CD (P=0.05)	1.13	2.17	1.32	2.72	4.15	0.836	0.332	5.64	0.096
Sem	0.376	2.042	0.532	2.72	3.81	2.83	1.40	4.47	0.096
CV	0.753	2.81	1.165	3.45	2.605	6.603	3.00	1.554	0.045

ha, respectively (Table 2). The increase in number of branches per plant with increase in N rate may be due to the fact that N promoted vegetative growth and branching (Ozturk, 2010).The interaction of spacing and N-levels was significant on this trait at 5% level (Table 3). The maximum number of branches/ plant (18.98) was obtained at the spacing of 30X15cm with the application of 120kg N/ha. These results confirms the findings of Ahmadi, 2010 that number of branches/plant significantly ($P<0.05$) increased with N doses from 0 to 150 kg/ha in *brassica* and may be attributed to increase in absorption and translocation of assimilates and stimulating apical and lateral meristem to grow.

Plant height

According to results, different spacing showed significant differences at 5% probability level (Table 1). Table 2, reveals that the 30X15cm spacing results in maximum height (66.24cm) of plants compared to 30X10cm (62.77cm). This was mainly due to higher plant population in closer spacing that might be attributed to reduction in magnitude of competition for light at closer spacing as compared to wider spacing leading to taller plants (Keivanrad and Zandi, 2012).The effect of N on this trait was significant at 5% level (Table 2) too. Increase in plant height was observed with increase in N-fertilization. The nitrogen dose of 120 and 90kg/ha gave the maximum plant height (75.10 and 66.57cm, respectively), and the plants with 0kg N/ha gave the minimum height (56.17cm).In total, increase in N-fertilization doses positively influenced the plant height. Nitrogen is a major constituent of chlorophyll and protein and its adequate supply through urea encouraged the photosynthesis, thereby resulting in stem elongation i.e. height (Malidarreh, 2010; Rasool *et al.*, 2013).The interaction between spacing and N-levels was significant on this trait at 5% level (Table 3).The highest plant height (78.60) was obtained at the spacing of 30X15cm with the application of 120kg N/ha (Table 3).Thus, wider spacing along with maximum N forms congenial environment for plants to attain higher heights. Similar results have been observed by Majnoun-hosseini *et al.,* 2006 in rapeseed and Zandi *et al.,* 2011 on fenugreek.

Number of pods/plant

As the results showed, the effect of spacing on this trait was significant at 5% level, too (Table 1). The number of pods/ plant increased with spacing, so that the highest and lowest ones (111.37 and 84.28) were produced at 30X15 and 30X10cm, respectively (Table 2).There were significant differences among the pods/ plant of various N-doses applied at 5% level (Table 1).The highest number of pods/ plant (134.02) was observed at 30kg N/ha (Table 2). The increase in N-level above 30kg N/ha adversely affected the number of pods/plant and the lowest ones (51.00) were produced by the application of 120kg N/ha (Table 2). The interaction between the N-fertilization and spacing affected this trait at 5% level (Table 3).The maximum number of pods/plant (152.45) was produced at the 30X15cm with the application of 30kg N/ha (Table 3). Similar observations were recorded by Sidlauskas and Tarakanovas, 2004.

Number of seeds/pod

Table 3: The combined effect of spacing and nitrogen fertilization on growth, yield and oil of *C. sativa* (mean of two years)

Treatments with code	Flowering (days)	Pod formation (days)	Branch/ plant(No)	Plant height (cm)	Pod/ plant(No)	Seed/ pod(No)	1000 seed weight(gm)	Seed Yield (kg/ha)	Oil(%)
S1N1	47.38	56.65	7.00	56.89	102.00	10.35	0.842	788.19	34.23
S1N2	48.00	58.00	7.98	58.40	116.00	11.40	0.752	845.39	35.66
S1N3	48.00	58.20	8.60	63.00	97.40	10.16	0.899	706.35	34.90
S1N4	48.33	59.67	9.25	64.00	58.00	9.20	0.940	669.78	34.36
S1N5	49.00	60.00	10.19	71.60	48.00	9.00	0.980	625.65	34.29
S2N1	47.00	57.67	9.20	55.45	148.05	11.00	0.910	875.23	34.83
S2N2	48.67	61.33	11.25	59.80	152.45	11.80	0.808	910.47	36.10
S2N3	50.00	75.00	15.60	68.20	113.35	10.60	0.911	820.59	35.76
S2N4	50.66	79.06	16.81	69.15	89.00	10.16	0.952	780.97	35.62
S2N5	56.00	80.65	18.98	78.60	54.00	9.80	1.068	738.74	35.01
CD(P=0.05)	NS	0.201	0.860	3.85	4.410	NS	NS	7.98	0.152

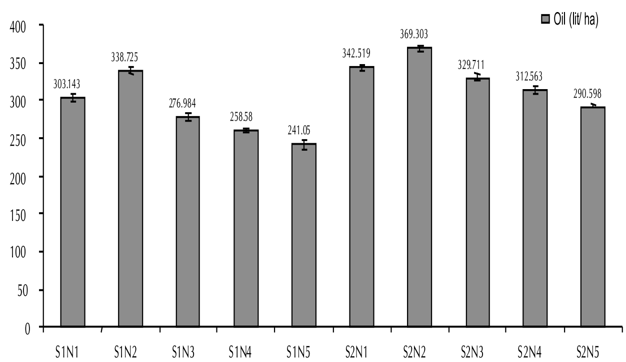

Figure 1: The combined effect of spacing and nitrogen fertilization on oil recovery of *Camelina*

Analysis indicated that the spacing does not affect the number of seeds/pod. The results are in line with Angadi *et al.,* 2003 and Danesh-shahraki *et al.,* 2008 findings that there is no link between plant population and number of seeds per siliqua. N-fertilization showed significant differences at P=0.05 in the number of seeds/pod (Table 1). The number of seeds/pod increased with increasing N rates upto N 30kg/ha. Thereafter, it declined slightly at the highest fertilizer rate of 120kg N/ha. Data showed that maximum seeds/pod (11.60) was recorded in those plots which received 30kg N/ha while the minimum (9.40) was produced by 120kg N/ha (Table 2). An adequate of N fertilizer enables the crop to produce rapid leaf growth which may positively contribute in seed filling. This is reflected in efficient partitioning of assimilate into economic yield resulting from the greater number of pods/plant and number of seeds/ pod (Al-Barrak, 2006). These results are in agreement with Ball *et al.*, 2000 for soybean and Fathi *et al.*, 2002, for colza. Vollmann, 1996; Wyosocki *et al.*, 2013; Sintim *et al.*, 2015 reported *Camelina,* as a low input crop compared to other oilseed crops. The interaction between spacing and N-fertilization was non significant on this character of *Camelina* at 5% level (Table 3).

1000 seeds weight

Results (Table 1) reveals, the spacing had significant differences on 1000-seed weight of *C sativa* cv Calena at 5% level (Table 2). Wider spacing of 30X15cm resulted in bolder seeds (0.929g/1000 seeds). The optimum density strengthened the optimal use of environmental condition for the crop and it lessened the inter plant competition which resulted in production of appropriate seeds with more weight. The effect of N on this trait was significant at 5% level too (Table 2).The highest and lowest thousand seed weight (1.024 and 0.946g) were produced by N levels of 120kg N/ha and 90kg N/ha, respectively (Table 2). Nitrogen fertilizer has been found to positively affect test seed weight of *brassica* (Mehmet, 2008). The interaction effect of spacing and nitrogen application does not affect this trait (Table 3).

Seed yield

According to results, (Table 1), different spacing showed significant differences at 5% probability level. The seed yield of 825.20kg/ha (Table 2) was produced at the spacing of 30X15cm and was high as compared to 30X10cm (727.07 kg/ha). With wider spacing seed number, biomass of single plant, test seed weight were the key factors to increase yield, and with narrow spacing there was a high intra-species competition, wasted resource and decrease of yield (Zhou et *al.*, 2011). The optimum plant density facilitates maximum utilization of nutrients and increased dry matter production which ultimately enhanced seed yield (Soomro, 2015). The effect of N on this trait was significant at 5% level, too (Table 2).The maximum and minimum seed yields (877.93 and 682.19kg/ha) were produced at N levels of 30kg N/ha and 120kg N/ha, respectively (Table 2). Further addition of N more than 30kg/ha cause the decrease of *Camelina* yield. The studies are supported by Wyosocki *et al.,* 2013 and Sintim *et al.,* 2015 that the basic nitrogen requirement of *C. sativa* is relatively low compared with other oilseed crops. The interaction between the N-fertilization and density affected this trait at 5% level (Table 3).The maximum seed yield (910.45kg/ha) was achieved at the 30X15cm spacing with the application of 30kg N/ha (Table 3). provided the optimum ground for obtaining the maximum level of the studied traits especially seed yield (Kardgar *et al.*, 2010). Similar results were observed by Majnoun-hosseini *et al.*, 2006; Dalal and Nandkar, 2011 that higher plant density and the addition of nitrogen could enhance *brassica* seed yield and its attributes.

Oil content of seeds

Results showed that there was no significant difference in oil content of seeds among different spacing (Table 1).The effect of N level on this trait was significant at 5% level (Table 1).The maximum and minimum oil contents (34.54 and 35.38%)

were observed in the seeds fertilized with 30kg N/ha and 0kg N/ha (Table 3). In the case of seed oil percentage, higher N levels result in lower seed oil content in *brassica* species as with the increase in N availability, more nitrogenous protein background are built and more proteins are used for building assimilates, so, fewer nutrients are available for synthesizing fatty acids (Brennan *et al.,* 2000; Saleem *et al.,* 2001).The interaction between the spacing and N-fertilization affected oil content of *Camelina* seed at 5% level (Table1) Similar findings were recorded by Cheema *et al.* 2001, that wider spacing and increasing rate of nitrogen negatively affected the oil contents, protein contents increased with increasing rate of N which showed inverse relationship with oil content in Canola.

In the current study, the maximum *C. sativa* seed oil (369.303 lit/ha) was obtained at the spacing of 30X15cm fertilized with of 30kg N/ha [Fig. 1; Results were shown as mean ± SD at CD (P=0.05)]. The higher oil yield in this interaction was also attributed to higher seed yield.

From the study, it is concluded that the spacing of 30X15cm with the fertilization of 30kg N/ha increases the productivity of *Camelina* and is thus recommended for large scale cultivation of *Camelina* as emerging biofuel crop in Central Western Himalayas of India.

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