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# Effect of Reduced Doses of Chemical Fertilizers with Dual Inoculation of Biofertilizers on Linseed Varieties

Leena Shakya<sup>1</sup>, \*Shubhra Barwa<sup>2</sup>

Assistant Professor, Dept. of Botany, Kirori Mal College, University of Delhi, India<sup>1</sup>

Assistant Professor, Bhaskaracharya College of Applied Sciences, University of Delhi, India<sup>2</sup>

\*Corresponding author

**ABSTRACT:** This study was conducted to work out the integrated use of fertilizers for the linseed varieties (Shubhra and Surabhi) on morphological, biochemical, yield and elemental composition. Two levels of the recommended dose of chemical fertilizers (reduced upto<sup>1</sup>/<sub>2</sub> and <sup>3</sup>/<sub>4</sub>) with dual inoculation of VAM and Azotobacter were applied along with full dose of chemical fertilizers. The growth and biochemical parameters were recorded at the pre-flowering, flowering and post-flowering stages and the yield-related characteristics at the harvest stage. The result reveals that both morphology and yield parameter produces a better result during the combination of biofertilizers and <sup>1</sup>/<sub>2</sub> dose of chemical fertilizers than full or reduced <sup>3</sup>/<sub>4</sub> dose of chemical fertilizers applied. Maximum increase in plant height, number of capsules m<sup>-2</sup> and seeds yield were recorded highest with the use of Azotobacter-inoculated seeds in combination with VAM and reduced dose of chemical fertilizers upto 50%.

KEYWORDS: Linseed, VAM, Azotobacter, compost and chemical fertilizers.

### I. INTRODUCTION

Linumusitatissimum, commonly known as linseed is one of the 100 species in the Linum genus belonging to Linaceae family. It is an important winter (Rabi) season oilseed and fiber-yielding crop of India and is important from industrial as well as medicinal point of view. It accumulates a high proportion of polyunsaturated fatty acids (PUFA) in its seed as "essential" fatty acids. Annual production of this crop is 40 lakh metric tons. The average national productivity (403 Kg ha-1) is also far below the world average productivity (851 kg ha-1), predominantly due to the susceptibility of the cultivars to biotic and abiotic stresses and also due to inadequate application of fertilizers and non-availability of nutrient from the soil. Selection of varieties based upon their efficiency in terms of production is important for the development of useful agro techniques.

Chemical fertilizers can only fasten the uptake of selective minerals thereby unbalancing the whole mineral pattern of plant body whereas biofertilizers has an ability to increase the amount of deficient nutrients in the soil. In combination with chemical fertilizers they help biological nitrogen fixation for the benefit of plants and fulfill the deficiency of phosphate content in soil. The fertilizer use efficiency has been very low in the Indian agriculture so to achieve sufficient nutrient level in the soil more amounts of chemical fertilizers has to be applied. In order to overcome the negative impact of excessive uses of chemical fertilizers there is a need to develop a suitable agricultural system which requires lower fertilizer input with higher fertilizer use efficiency. In view of the priority for the promotion of organic farming and reduction of chemical residues in the environment, special attention has to be given to the use of biofertilizers. Recently, Vesicular Arbuscular Mycorrhiza (VAM) and Azotobacter are the most widespread biofertilizers and they significantly contribute elemental content to plants and also provide resistance to drought situation However, very limited or no information is available regarding the effect of bio-fertilizers on linseed varieties.



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### **II. MATERIALS AND METHODS**

The authentic seeds of linseed varieties were obtained from Chandra Shekhar Azad (CSA) University, Kanpur, UP. Standard agricultural practices were employed for the cultivation. The seeds were sown in mid October in well-aerated, ploughed soil which was homogeneously mixed with recommended doses of fertilizers. The study was conducted in the experimental field of the Herbal Garden, JamiaHamdard,New Delhi. Before starting the experiments initial soil pH, organic carbon content, Cationic Exchange Capacity (CEC), available N, P and K were analyzed by the Soil Testing Laboratory, Division of Soil Science and Agricultural Chemistry, IARI, New Delhi. The relevant physicochemical properties of soils were: pH 7.9, organic carbon 0.47%, EC 0.03 m mhos cm<sup>-1</sup>, available N 128 kg ha<sup>-1</sup>, available phosphorus 24.35 kg ha<sup>-1</sup> and available K 109 kg ha<sup>-1</sup>. Before sowing in each plot, the fertilizers were applied. Chemical fertilizers were applied directly to the soil whereas biofertilizers were applied to the seed by seed inoculation and soil by layering method respectively. The sources of N, P, K, S, and Zn were urea, single super-phosphate, muriate of potash, calcium sulphate and zinc sulphate respectively.

The biofertilizers used in the study were culture of *Vesicular Arbuscular Mycorrhizae* (VAM) and *Azotobacter chroococcum*, procured from The Division of Microbiology, Indian Agricultural Research Institute (IARI), New Delhiand National Biofertilizer Development Centre (NBDC), Ghaziabad, Uttar Pradesh respectively. They were applied through soil (layering method) and seed (seed inoculation method) respectively. The treatments used: were T<sub>0</sub>=Control, T<sub>1</sub>=Full dose of Recommended Chemical Fertilizer i.e. N @ 30 kg ha<sup>-1</sup>, P @ 20 kg ha<sup>-1</sup>. K@80 kg ha<sup>-1</sup>, S@120 kg ha<sup>-1</sup>, Zn@26 kg ha<sup>-1</sup>, T<sub>2</sub>=Reduced 3/4 dose of chemical fertilizers with biofertilizers (dual inoculation with *Azotobacter* and VAM), T<sub>3</sub>=½ dose of chemical fertilizers with biofertilizers (dual inoculation with *Azotobacter* and VAM). Basal dose of S, K and Zn @ 26, 90, 10 Kg ha<sup>-1</sup> respectively were applied to all treatments at sowing time.

Samplings for growth and biochemical parameters were done at preflowering, flowering and post-flowering stage and yield characteristics were recorded at the time of harvest. Physiological parameters were conducted in the intact leaves. Yield characteristics were noted at harvesting. Elemental analysis was done after drying the plant part.

To observe and study the effect on growth and developments of linseed plant, following parameters were studied.

### Morphological parameters

The plant height was measured in centimetres (cm). Plants were removed from the soil and divided into root, stem and leaves. Capsules were separated from the shoot part at the junction of the peduncle and stem. The separated plant parts were weighed as fresh weight (f.w.) and then dried separately in a hot air oven at  $65^{\circ}\pm2^{\circ}$ C for 48 hours. Weights of the samples were recorded with the help of an electronic balance (Sartorius-GE412).

### Physiological characteristics

Total Chlorophyll content in the leaf was estimated in fresh leaf samples by the method [12].

#### Plant analysis for elemental composition

Leafy part of the Plant was analyzed for nitrogen (N) and potassium (K) status. Three plants were collected from each pot at each sampling andwere wiped for making it free of any adhering dust. They were first washed in running tap water for 1 min followed by 1 min in distilled water. Each sample was dried for 48 h in hot air oven at 65°C. The dried samples were fine powdered and passed through a 72 mm mesh screen. The powder was stored in labelled polyethylene bags and used for analysis.

The nitrogen content (% dry weight) was analyzed by packing the known weight of plant sample-powder in tin boats with the help of Elementar system (CHNS Analyzer, Vario EL.) in its CHNS mode. Potassium content was analysed using flame photometry method.

### **Yield related components**

The harvested samples were sun-dried and threshed manually. Yield parameters namelyseed yield (kg ha-1), 1000 seed weight (g) and Oil content (%) were recorded for each replicate Fixed oil of the dried seeds was extracted by cold maceration [14].



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Data were statistically analyzed using analysis of variance (ANOVA) by SPSS (Statistical Procedure for Social Sciences, ver. 11.0 Inc., Chicago, USA). The Critical Difference (CD) was calculated for the significant data at P < 0.05.

### III. RESULTS AND DISCUSSION

Plant fresh weight Fresh weights of the plants have increased progressively and have attained maximum value at postflowering stage (Table 1). Dual inoculation by VAM and Azotobacter has significantly affected the plant height in both the varieties of linseed at the reduced doses of chemical fertilizers. Maximum percent variation has been observed in Surabhi (60.58%) over the control than Shubhra at pre-flowering stage.

#### Plant height

The differences in plant height among varieties and treatments have been found significant. Maximum percent variation over control has been observed at Surabhi at T3 treatment in pre-flowering stage (62.28%). The interactive effects of varieties and fertilizers treatments have been found significant at all the growth stages (Table 1). However, at flowering stage, there is much significant difference and has been shown by the treatments. Shubhrahas responded minimum to the treatments applied. On an average, with treatment having reduced dose of chemical fertilizer upto 50% when supplementing with biofertilizer (T3) has shown better response over the full dose of chemical fertilizers (T1).

### Plant dry weight

Dry mass has shown a significant increment at all the three stages of plant growth. An overall significant enhancement has been observed in Surabhi (Table 1) at T3 treatment in comparison with control. The reduction in chemical fertilizers has proved to be most effective in increasing the plant dry weight. Increase in percent variation over control has been observed to be highest in Surabhi at all the stages. Clear variations have been found in dry matter plant-1 with different levels of chemical fertilizers among the growth stages. Plants well supplied with nutrients produced more leaf matter, resulting in an increased in CO2 assimilation by the plant. At flowering stage, the dual inoculation with 50% recommended dose of chemical fertilizers produced the highest dry matter over the 3/4 and no reduction. In the present study, inoculated Surabhi variety has shown significant increase in dry biomass over non-inoculated plant. This enhancement by the biofertilizers was also reported by [3].

### Chlorophyll content

Chemical fertilizers when supplementing by inoculating with biofertilizers has significantly affected physiological characteristics of the crop. Dual inoculation by VAM and biofertilizers has resulted in significant improvement in the total chlorophyll content as has been compared with the control at all stages of plant growth (Table 2). Considerably higher total chlorophyll has been noted at flowering stage. In general, reduction in fertilizer doses upto 50% has resulted in enhancement in the total chlorophyll content from 2.278 to 3.402 mg g<sup>-1</sup> fresh weight at the flowering stage in Surabhi. Enhancement has also been observed in all other treatments but they are found less significant than T3 treatments. Surabhi showed high chlorophyll content as compare to Shubhra. Regarding treatments 50% reduction in doses of chemical fertilizers along with biofertilizers proved to be most effective one. Most of the researchers have suggested that VAM symbiosis increased the units of photosynthesis, and so as to increase the rates of photosynthetic storage and export at the same time[1]. It has been proved that concentration of chlorophyll in VAM plants was higher than their control plants [16] resulting in higher photosynthesis rate [6] which is further boosted by the addition of *Azotobacter* in dual inoculation.

### Potassium (K) content

The potassium content has been found to increase significantly with the application of fertilizers (Table 3). Maximum improvement has been observed in Surabhi. It has shown an increase of about 49.53% over their respective controls. Varietal differences have also been found significant.



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#### Nitrogen content

The trend of plant response to the treatments regarding N content has increased significantly in varieties and treatments (Table 3). Maximum N content has been observed in Surabhi (4.13%), which has increased up to 4.84 % dry weight with T3 treatment. Inoculating plants with VAM + Azotobacter + 75% N P has resulted in the increased elemental content, but the highest percentages have been obtained by fertilizing the plants with a mixture of VAM +Azotobacter + 50% NP. This could be due to the effect of proper establishment of the microbes in 50% dose of chemical fertilizers. Soil microorganisms that colonize the rhizosphere assist plants in the uptake of several vital nutrients, such as K and N, from soil [5]. It has been reported that nitrogen, and potassium content in leaves of *Foeniculum* vulgare and *Antheumgraveolens* [10] have increased by applying fertilizers. The tissue nitrogen content significantly increased over non-inoculated plant. This enhancement by the biofertilizers has also been reported by [11]. The present study has shown an increase in nutrient content especially nitrogen (N) and potassium (K). Our findings are in concurrence with [9], who has reported that VAM hyphae improved the capacity of higher plants to acquire inorganic nitrogen. Uptake and transport of N through VAM external hyphae has been reported [13]. It has been observed that VAM inoculation significantly increased the potassium content in tamarind seedlings[15]. Enhancement in yield attributes would ultimately culminate into seed-yield production of linseed variety. Seed-yield is a cumulative performance of capsule number, seed number per capsule and 1000-seed weight.

#### Capsule/plant

Significant difference in terms of the response of both varieties to treatments, their interactive effects (VxT) have been observed for number of capsules per plant (Table 3). Significant increase in number of capsules has been observed with treatment applied. The T3 treatment has resulted in higher increment in weight of capsule than compared to other treatments (32.18%). Reducing the dose up to 50% has proved to be the most effective as compared to 75% reduction when supplied with dual inoculation of biofertilizers.

#### 1000 seed weight (g)

The effect of fertilizer application has been found highly significant towards 1000 seed weight in the present experiment which was seen maximum in Shubhra upon the treatment with 50% of reduced doses of chemical fertilizer in combination with biofertilizer (Table 3). The results are in line with [20] on Brassica cultivars. The results suggest distinct changes in seed weight and quality in the presence of added chemical fertilizers; thus, confirming the findings of [17] and [4] in cotton.

#### Oil content (%)

Irrespective of varietal differences, oil content has been found highest in Surabhi (48.8%) at T3 treatment (Table 3). A significant varietal difference has been observed in oil content at harvest. Oil yield in both the varieties has shown positive response to treatment applied in comparison to control. In Surabhi the same treatments that earlier proved superior for growth resulted in the production of maximum oil yield. Thus, the treatment containing dual inoculation of biofertilizers with 50% reduced dose of chemical fertilizers proved to be the best and it increased the oil yield over Shubhra which gave the lowest value. Similar findings on oil content have been shown by [19] in mustard crop. [2] and [7]have found that fennel responded to biofertilizers by increasing growth and oil yield. A significant increase in the oil content of inoculated seeds (*Azotobacter* and *Azospirillum*) has been reported [8] in*Ammivisnaga*.



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## IV. TABLES

Table 1.Effect of reduced doses of chemical fertilizers with biofertilizers on growth characteristics of linseed varieties at three growth stages.

Treatment	Pre-flowering stage			Flowering stage			Post-flowering stage					
s	SHUBHRA	SURABHI	Mean	SHUBHRA	SURABHI	Mean	SHUBHRA	SURAE	BHI Mean			
	$(V_1)$	$(V_2)$		$(V_1)$	$(V_2)$		$(V_1)$	$(V_2)$				
Plant fresh weight (g plant <sup>-1</sup> )												
$T_0$	2.74	2.38	2.56	46.98	44.23	45.60	91.34	50	.28 70.81			
$T_1$	2.87	2.82	2.84	54.59	53.81	54.20	57.54		7.54 77.01			
	(4.86)	(18.26)		(16.20)	(21.67)		96.49 (5.64) (14.42)		4.42)			
T <sub>2</sub>	2.92	2.91	2.92	59.16	57.56	58.36	65.94		<sup>5.94</sup> 81.46			
	(6.57)	(22.29)		(25.93)	(30.14)		96.98 (6.17) (31.13					
T <sub>3</sub>	3.11	3.83	3.47	61.98	65.92	63.95	73.92		<sup>3.92</sup> 01 74			
	(13.55)	(60.58)		(31.93)	(49.06)		109.57 (19.96) (47.00		7.00) 91.74			
Mean	2.91	2.98		55.68	55.38		98.59 61.92		.92			
C.D.	V=NS	T=0.41 V	xT=NS	V=NS	T=4.28	VxT=NS	V=3.58	T=5.06	VxT=NS			
(5%)												
Plant height (cm)												
T <sub>0</sub>	11.4	10.8	11.11	51.4	30.4	40.52	60.9	32.8	46.85			
T <sub>1</sub>	12.3	12.9	12.60	63.1	32.1	47.59	65.6	35.8	50.67			
	(7.14)	(20.07)		(22.60)	(5.61)		(7.60)	(9.20)				
$T_2$	19.9	12.4	16.15	68.3	34.9	51.59	70.6	37.3	53.98			
	(73.86)	(15.12)		(32.81)	(14.69)		(15.90) (13.94)					
T <sub>3</sub>	18.1	17.5	17.80	75.9	37.5	56.71	82.8	39.9	61.38			
	(58.27)	(62.28)		(47.52)	(23.46)		(35.91)	(21.87)				
Mean	15.42	13.40		64.67	33.73		69.98	36.46				
C.D.	V=0.87	T=1.23	VxT=1.	V=2.24	T=3.17	VxT=4.49	V=1.73	T=2.44	VxT=3.46			
(5%)			74									
Plant dry weight (g plant <sup>-1</sup> )												
T <sub>0</sub>	0.23	0.22	0.23	9.51	9.41	9.46	12.71	12.84	12.78			
$T_1$	0.25	0.29	0.27	11.37	9.78	10.57	14.50	13.02	13.76			
	(6.06)	(32.73)		(19.55)	(3.91)		(14.07)	(1.37)				
T <sub>2</sub>	0.29	0.30	0.29	12.05	12.69	12.37	15.13	14.61	14.87			
	(24.24)	(37.27)		(26.69)	(34.90)		(19.03)	(13.74)				
T <sub>3</sub>	0.29	0.44	0.37	14.51	13.74	14.13	16.06	16.23	16.14			
	(25.97)	(101.36)		(52.62)	(46.02)		(26.31)	(26.37)				
Mean	0.26	0.31		11.86	11.40		14.60	14.17				



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Table 2.Effect of reduced doses of chemical fertilizers with biofertilizers on total chlorophyll content in leaves of SHUBHRA and SURABHI of linseed at three growth stages.

Treatments	Pre-flowering stage			Fl	owering sta	ge	Post-flowering stage			
	SHUBHRA	SURABHI	Mean	SHUBHRA	SURABH	I Mean	SHUBHRA	SURABHI	Mean	
	$(V_1)$	$(V_2)$		$(V_1)$	$(V_2)$		$(V_1)$	(V <sub>2</sub> )		
Total chlorophyll content (mg $g^{-1}$ fw)										
$T_0$			0.85			2.13			1.3	
	0.711	0.981		1.983	2.278		1.141	1.458	0	
$T_1$	0.728	1.061	0.89	2.048	2.771	2.41		1.505	1.4	
	(2.39)	(8.15)		(3.28)	(21.64)		1.296 (13.58	3) (3.22)	0	
$T_2$	0.819	1.114	0.97	2.070	2.835	2.45		1.518	1.4	
	(15.19)	(13.56)		(4.39)	(24.45)		1.331 (16.65	5) (4.12)	2	
T <sub>3</sub>			1.06			2.79		1.802	1.6	
	0.840	1.272		2.171	3.402			(23.59	5	
	(18.14)	(29.66)		(9.48)	(49.34)		1.477 (29.45	5) )		
Mean	0.77	1.11		2.07	2.82		1.31	1.57		
C.D.	V=0.054	T=0.0.077	VxT=	V=0.049	T=0.035	VxT=0.070	V=0.030 T=	0.0.04 VxT	=0.060	
(5%)	NS						2			

 Table3. Effect of reduced doses of chemical fertilizers with biofertilizers on yield characteristics and elemental composition of SHUBHRA and SURABHI of linseed at the harvesting stage.

		Variety		Variety						
Treatments	SHUBHRA	SURABHI	Mean	SHUBHRA	SURABHI	Mean				
	(V <sub>1</sub> )	(V <sub>2</sub> )		(V <sub>1</sub> )	(V <sub>2</sub> )					
	100	0 seed weight	(g)	Ca	Capsule Plant <sup>-1</sup>			Oil content (%)		
TO	1.00	22.3	22.3	22.3	26.6	20.26	30.3	44.1	37.23	
T1						24.12	31.1	45.8	38.47	
	1.07 (13.96)	23.3 (4.48)	23.3 (4.48)	23.3 (4.48)	32.2(20.56)		(2.62)	(3.84)		
T2						32.27	32.6	48.5	40.54	
	1.21 (20.74)	24.0 (7.46)	24.0 (7.46)	24.0 (7.46)	45.4 (70.76)		(7.40)	(9.95)		
Т3						36.95	32.7	48.8	40.72	
	1.25 (24.79)	24.3 (8.96)	24.3 (8.96)	24.3 (8.96)	49.1 (84.80)		(7.69)	(10.57)		
Mean	1.13	23.50	23.50	23.50	38.25		31.67	46.81		
C.D. (5%)	V=0.01	V=1.81	V=1.81	V=1.81	T=4.06	VxT=5.7	1.44	T=2.04	VxT=N	
	1	N content (%)		K	K content (%)				~	
T <sub>0</sub>	4.00	4.13	4.06	1.0	2.0	1.52				
$T_1$	4.01 (0.30)	4.28 (3.73)	4.15	1.1 (8.83)	2.3 (13.27)	1.70				
$T_2$	4.11 (2.70)	4.78 (15.80)	4.44	1.5 (42.39)	2.7 (32.29)	2.06				
<b>T</b> <sub>3</sub>	4.13 (3.17)	4.84 (17.25)	4.48	1.5 (43.47)	3.0 (49.53)	2.24				
Mean	4.06	4.51		1.26	2.50					
C.D. (5%)	V=0.22	T=0.31	VxT=NS	V=0.213	T=0.310	VxT=				



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#### V. CONCLUSION

Seed yield is a direct representation of the amount of dry matter accumulated during the growing season and its partitioning into seeds.[18] have reported an increase in seed yield with increasing nitrogen rates. The comparatively higher yields and yield quality attributes were obtained from reduction in chemical fertilizers (50%) with biofertilizers. The study shows that yield components of linseed have been affected significantly by inoculation with Azotobacter and VAM, because biofertilizers can fix nitrogen and solubilise phosphorus in the soil and enhance absorbance of elements by the plant. So they proved to be the best supplement for chemical fertilizers.

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