

Spacing, fertilizer and PGPR on yield and nutrient uptake in industrial hemp (*Cannabis sativa* ssp. *sativa*) and chemical properties of soil

ABSTRACT

A field experiment was conducted to assess the influence of spacing, fertilizer levels and PGPR on yield and primary nutrients accumulation in different parts of industrial hemp and on chemical properties of soil. The experiment was laid out in Factorial Randomized Complete Block Design, spacing and fertilizers levels with or without PGPR were the factors. The results revealed, plants spaced at 15cm × 10cm registered significant yield of leaves, stems and roots (7.84, 9.80 and 4.20 t ha⁻¹ respectively). The uptake of nitrogen and potassium (450.32 and 159.13 kg ha⁻¹ respectively) was highest in this spacing, while, the phosphorous uptake (75.74 kg ha⁻¹) was maximum in 10cm × 5cm spacing. Among nutrition, 125 percent RDF plus PGPR recorded a significant yield of stem and seed, also, this treatment resulted in maximum uptake of nitrogen and phosphorus (440.33 and 72.58 kg ha⁻¹ respectively) by the plants. It can be inferred that spacing of 15cm × 10cm and application of 10 tonnes FYM with 250:125:250 kg N, P₂O₅, K₂O ha⁻¹ (125% RDF) plus PGPR consortia (*Azospirillum brasilense*, *Pseudomonas fluorescens* and *Bacillus megaterium* @ 5kg each) was found to be optimum to produce higher yields.

Key words: Hemp, Spacing, Nutrition, PGPR consortia and Nutrient uptake.

1. INTRODUCTION

Industrial hemp (*Cannabis sativa* ssp. *sativa*) is a member of the Cannabaceae family, native to Western and Central Asia. It had spread to the whole world in post Columbian times and is under cultivation in many parts of the world for more than 10,000 years for medicinal, fiber, seed and recreational purposes [3]. The medicinal use of hemp has been accompanied by discovery of

substances Cannabidiol (CBD) and Tetrahydrocannabinol (THC). It is globally now being cultivated in an area of 1.05 lakh hectares and China, North Korea, Chile, France and Germany are the five biggest hemp-producing countries [22]. Hemp is a dual-purpose crop where both fibers and seeds have economic importance. The stem of hemp is erect with an outer bark that contains long tough bast fibers made up of 70 per cent cellulose, 22 per cent hemicellulose and 6 per cent lignin which make the fibers as earth's longest and strongest natural fiber [18] having industrial importance.

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Plants need nutrition throughout their growing period for various physiological functions and each element has a specific role to perform in plant metabolism. Among 17 essential nutrients, Nitrogen, Phosphorus and Potassium plays a vital role in plant growth and metabolism. Shreds of research evidences have shown the importance of macro and micronutrients in plant growth and yield and their imbalanced nutrition will have bad have negative effects on growth, yield and quality. The supply of appropriate nutrition is vital aspect in production of plants having commercial importance [7]. Therefore, assessment and supply of nutrients in appropriate quantities is a requirement of any crop and it is more so in industrial crops like hemp, since it can affect the plant biomass production, seed yield and fiber yield and as well as quality. Hemp requires application of high amounts of nutrients as most of the supplied nutrients return to the soil through the leaf fall and decomposition via senescence of leaves during growth period. Hence, nutrients concentration in hemp could be an important diagnostic tool for the optimization of mineral fertilization [4]. A good nutrition package having chemical and biological means via the addition of chemical fertilizers and plant growth-promoting rhizobacteria (PGPR) to increase fertilizer use efficiency could have positive effect on crop productivity and ecosystem functioning. PGPR can promote plant growth and development through several mechanisms like atmospheric nitrogen fixation, siderophores production, minerals solubilization and production of plant hormones [21]. The limited scientific information available on the cultivation of hemp crop in Indian soils could be a limiting factor to achieve good productivity, therefore efforts have been made to optimize investigate the impact of spacing and nutrition package for industrial hemp crop, particularly for the Eastern dry zone of Karnataka with an objective to enhance the productivity of hemp.

2. MATERIAL AND METHODS

Field experiment was conducted at the College of Horticulture, UHS campus, Bengaluru, Karnataka, India during *Kharif* 2019-20 to standardize the suitable plant spacing and optimum

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nutrition for industrial hemp crop. The soil of the experimental site was red sandy loam (37% sand, 8% silt and 17% clay) in texture, neutral (6.57) in reaction and low in total soluble salts (0.13 dS m⁻¹) and organic carbon (0.41%) contents but medium in available primary nutrients status (313.6, 39.50 and 187.76 kg N, P₂O₅, K₂O ha⁻¹, respectively).

2.1 Experimental details

The experiment was laid out in Two Factorial Randomized Complete Block Design with twenty-four treatment combinations replicated thrice by considering the plant spacing as one factor and nutrition with or without PGPR consortia as another factor. Plots of one meter square were amended with well decomposed FYM @ 10 tons per hectare and treatments imposed. One week old seedlings of NHEMPCO Vijaya-I fiber strain were transplanted in a plot size of one square meter at six different spacings viz., S₁: 10 cm × 5 cm (200 plants m⁻²), S₂: 10 cm × 10 cm (100 plants m⁻²), S₃: 15 cm × 5 cm (133 plants m⁻²), S₄: 15 cm × 10 cm (66 plants m⁻²), S₅: 20 cm × 5 cm (100 plants m⁻²) and S₆: 20 cm × 10 cm (50 plants m⁻²) and applied with four different levels of nutrition viz., N₁: 10 t FYM ha⁻¹ + 150:75:150 kg N, P₂O₅, K₂O ha⁻¹ (75% RDF) + PGPR consortia, N₂: 10 t FYM ha⁻¹ + 200:100:200 kg N, P₂O₅, K₂O ha⁻¹ (100% RDF) + PGPR consortia, N₃: 10 t FYM ha⁻¹ + 250:125:250 kg N, P₂O₅, K₂O ha⁻¹ (125% RDF) + PGPR consortia and N₄: FYM 10 t ha⁻¹ + 200:100:200 kg N, P₂O₅, K₂O ha⁻¹ (100% RDF) without PGPR consortia (Control). The PGPR consortia consisting of *Azospirillum brasilense*, *Bacillus megaterium* and *Pseudomonas fluorescens* were added 10 days after transplanting as per the treatments at the rate of five kilo gram each. The levels of fertilizers as per treatment were applied in the form of urea, single super phosphate and muriate of potash. Fifty per cent of nitrogen and a full dose of phosphorus and potassium were applied 15 days after transplanting and the remaining fifty per cent of nitrogen was applied 30 days after transplanting. Irrigation, weeding and other crop management practices were followed as and when required throughout the crop growth period.

2.2 Yield parameters (t ha⁻¹)

All the inflorescences from each plot were harvested separately, shade dried, threshed and collected. Seed yield was expressed in tonnes per hectare by extrapolating to yield per plot. After seed harvest, plants were uprooted plot wise separately, roots and leaves separated from

stems, shade dried to record the dry weight of leaves, stems, seeds, roots and dry weights expressed in tonnes per hectare.

2.3 Plant and soil analysis

At harvest, five randomly selected plants were uprooted from each treatment plot separately. The leaves, stems, roots and seeds were separated, oven dried at 65°C, ground separately to a fine powder using a Willey mill. Phosphorous content was estimated by vanadomolybdate yellow color method as described by [23], potassium by flame photometric method and total nitrogen content by Macro-kjeldahl's digestion and distillation method [23]. The total N, P and K uptake by different parts of the hemp crop was calculated by multiplying the dry yield of hemp parts with their respective nutrient contents and expressed in kilograms per hectare.

For Residual soil nutrient analysis after crop harvest was done. Soil samples were collected from 0-30 cm depth from each treatment plot separately after the harvest of the crop and were processed for further analysis as described by Jackson [14]. The soil pH, electrical conductivity (EC) and organic carbon content were analyzed [14]. The available nitrogen content of the soil was determined by adopting alkaline potassium permanganate method in Kjeldahl's distillation unit as outlined by Subbiah and Asija [32]. The available phosphorus content of the soil was extracted using either Bray's extractant-I or Olsen's extractant depending on the pH of the sample. Further, phosphorus in the extract was determined by chlorostannus reduced molybdophosphoric blue color method in the HCl system [14]. The available potassium content of the soil was extracted with neutral normal ammonium acetate solution and estimated by a flame photometric method using a suitable filter [14]. The experimental data obtained were subjected to statistical analysis by using ICAR-WASP 2.0 programme developed by ICAR Research Complex, Goa. Wherever the F-test was significant, LSD values were worked out at 0.05 probability level for the comparison of treatment means.

3. RESULTS AND DISCUSSION

3.1 Biomass yield of different hemp parts

Hemp plants spaced at 15 cm × 10 cm, 10 cm × 10 cm and 10 cm × 5 cm registered significantly maximum yields of leaves (7.84 t ha⁻¹), stems (9.80 t ha⁻¹) and roots (4.20 t ha⁻¹). Minimum leaf yield of 6.08 t ha⁻¹ was recorded in plants spaced at 10 cm × 5 cm while, a minimum

stem yield of 6.87 t ha⁻¹ and root yield of 1.87 t ha⁻¹ were noticed in plants spaced at 20 cm × 10 cm (Table 1). However, seed yield was not influenced by the different spacings. With regard to nutritions tested, plants provided with 125 per cent RDF plus PGPR consortia recorded significantly maximum yield of stem (9.93 t ha⁻¹) and seed (6.83 t ha⁻¹). While, minimum stem (5.72 t ha⁻¹) and seed (4.59 t ha⁻¹) yields were recorded in control. However, the nutrition did not show any significant differences in leaf and root yields.

Among the interactions, plants spaced at 15 cm × 10 cm and supplied with only 100 per cent RDF recorded significantly maximum leaf yield (9.57 t ha⁻¹) while, maximum yield of the stem (12.35 t ha⁻¹) and seed (8.39 t ha⁻¹) were registered from plants spaced at 10 cm × 10 cm and supplied with 125 per cent RDF with PGPR consortia. Plants spaced at 10 cm × 5 cm and supplied with 75 per cent RDF with PGPR consortia recorded significantly maximum root yield (6.23 t ha⁻¹). Lowest leaf, stem and seed yields were recorded in plants that received only 100 per cent RDF without PGPR consortia and spaced at 10 cm × 5 cm (Table 1). Significantly lowest root yield was obtained in plants that received only 100 per cent RDF without PGPR consortia and spaced at 20 cm × 5 cm indicating the beneficial effects of biofertilizers in hemp cultivation. The Highest yield observed in 10 cm × 10 cm spacing and 125 per cent RDF with PGPR consortia suggests it to be an optimum spacing and nutrition. It would have provided sufficient area for sunlight penetration and photosynthesis coupled with the maximum availability of nutrients applied in the form of inorganic fertilizers along with biofertilizers and FYM [11]. This results in favorable effects on the physical, chemical and biological properties of the soil thereby supplying required nutrients in their available form bythrough increasing the microbial population in the soil by providing sufficient energy for them to remain active [33].

Table 1. Influence of spacing and nutrition on yield of different hemp parts (t ha⁻¹)

Treatments	Leaves	Stems	Seeds	Roots
S ₁	6.08	8.45	5.68	4.20
S ₂	6.87	9.80	5.79	2.50
S ₃	6.34	8.18	5.83	2.95
S ₄	7.84	7.86	6.01	2.67
S ₅	7.28	8.32	6.11	2.72
S ₆	6.26	6.87	5.24	1.87
S.Em ±	0.49	0.77	0.27	0.39
CD at 5%	1.43	2.25	NS	1.14
N ₁	7.36	8.70	5.83	3.31
N ₂	6.34	8.65	5.86	2.78
N ₃	7.13	9.93	6.83	2.99
N ₄	6.27	5.72	4.59	2.19
S.Em ±	0.40	0.63	0.22	0.32
CD at 5%	NS	1.85	0.65	NS

S ₁ N ₁	6.25	8.43	5.45	6.23
S ₁ N ₂	6.27	8.35	5.59	2.68
S ₁ N ₃	7.65	11.88	8.07	4.09
S ₁ N ₄	4.14	5.15	3.62	3.80
S ₂ N ₁	8.82	10.30	5.47	2.96
S ₂ N ₂	5.40	10.80	4.75	2.64
S ₂ N ₃	8.82	12.35	8.39	2.78
S ₂ N ₄	4.45	5.75	4.55	1.63
S ₃ N ₁	6.89	8.50	5.53	2.54
S ₃ N ₂	6.36	8.75	6.58	3.49
S ₃ N ₃	7.30	10.83	7.15	2.57
S ₃ N ₄	4.80	4.65	4.09	3.19
S ₄ N ₁	8.27	7.50	5.97	2.30
S ₄ N ₂	7.52	8.53	7.72	3.17
S ₄ N ₃	5.99	8.05	4.68	3.71
S ₄ N ₄	9.57	7.35	5.66	1.50
S ₅ N ₁	7.22	10.83	7.28	3.67
S ₅ N ₂	6.91	7.53	5.28	2.64
S ₅ N ₃	7.16	8.93	7.48	3.32
S ₅ N ₄	7.82	6.00	4.40	1.25
S ₆ N ₁	6.73	6.63	5.29	2.19
S ₆ N ₂	5.61	7.93	5.25	2.08
S ₆ N ₃	5.86	7.53	5.21	1.44
S ₆ N ₄	6.82	5.40	5.20	1.78
S.Em ±	0.98	1.54	0.54	0.77
CD at 5%	2.85	4.50	1.59	2.26

3.2 Primary nutrients content in different parts of hemp plant

The data on NPK content in different parts of hemp crop as influenced by various spacing and nutrient management practices, presented in Table 2 indicates that significantly highest nitrogen content, 2.51% in leaves and 3.53% in seeds were observed in the plants spaced at 15 cm × 10. The stem (0.43%) and root (0.83%) nitrogen was found highest in the spacing, 15 cm × 5 cm and 20 cm × 10 cm respectively. The variation in nitrogen content of different parts suggests the translocation and accumulation of nitrogen absorbed from soil to actively growing parts of the plant such as leaves and seeds. It was possible due to the optimum spacing, less competition for space and more availability of light and nutrients to the plants resulting in increased metabolic activity and growth of plants and chlorophyll content of leaves [30]. Increased chlorophyll content

NS: Non significant
S₁: 10 cm × 5 cm
S₂: 10 cm × 10 cm
S₃: 15 cm × 5 cm
S₄: 15 cm × 10 cm
S₅: 20 cm × 5 cm
S₆: 20 cm × 10 cm
N₁: 75% RDF + PGPR consortia
N₂: 100% RDF + PGPR consortia
N₃: 125% RDF + PGPR consortia
N₄: 100% RDF

inflicts better photosynthetic rate and facilitates more uptake of nitrogen from the soil and

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accumulation in different plant parts [34, 24]. Among nutrition, hemp plants nourished with 75 per cent RDF plus PGPR consortia recorded the highest nitrogen content in leaves (2.57%), stems (0.38%) and seeds (3.38%), while root nitrogen content (0.83%) was highest in plants applied with 100 per cent RDF alone. Biofertilizer, *Azospirillum* ~~was-is~~ known to fix atmospheric nitrogen up to 40kg per hectare per year in addition to production of plant growth promoters at the root interface, which might have stimulated root development and supplied nitrogen thereby increasing the nitrogen uptake and concentration in plants [20, 2]. ~~Other PGPR also would have contributed for plant growth.~~

~~If interaction is considered~~Considering interaction., hemp plants spaced at 10 cm × 5 cm and supplied with 125 per cent RDF plus PGPR consortia recorded significantly maximum nitrogen content (3.00%) in leaves. ~~Whereas, p~~Plants spaced at 15 cm × 5 cm spacing and applied with 100 per cent RDF alone recorded significantly maximum nitrogen content in stems (0.48%) and seeds (4.01%). But plants which received 75 per cent RDF plus PGPR consortia and spaced at 20 cm × 10 cm recorded significantly maximum nitrogen content (1.05%) in roots. The probable reason for increased nitrogen content might be due to good growth of plant at optimum spacing and mineralization of organic nitrogen to available form due to biofertilizers application when compared to narrow spacing and application of inorganic fertilizers alone [16, 34].

The phosphorus content in stems and seeds of hemp differed significantly while, its content in leaves and roots did not differ significantly due to different spacings tested (Table 2). Plants spaced at closer spacing of 10 cm × 5 cm recorded significantly highest phosphorus content in stems (0.09%) and seeds (0.66%). This might be due to phosphorus ~~is-being~~ highly immobile in soil and ~~it-is~~ taken up by plant roots only by direct root contact mechanism. In closer spacings, plant roots were in contact with larger soil surface that resulted in higher uptake of phosphorus [27]. The phosphorus content in different parts of hemp except seeds differed significantly due to nutrition treatments tested. Hemp plants which received 100 per cent RDF plus PGPR consortia recorded significantly maximum phosphorus content in leaves (0.42%) and roots (0.13%). However, application of 100 per cent RDF alone recorded significantly maximum phosphorus content (0.09%) in stems. Addition of *Pseudomonas* might have promoted root growth and making the plant roots in contact with larger soil surface [16]. Further, microorganisms help in synthesis of siderophores which play a key role in solubilization of phosphate minerals and helps in increasing phosphorus uptake [9, 2]. Plants spaced at 10 cm × 5 cm and provided with 100 per

cent RDF plus PGPR consortia recorded significantly maximum phosphorus content (0.85%) in leaves. Whereas, plants spaced at 10 cm × 5 cm and applied with 125 per cent RDF plus PGPR consortia recorded maximum phosphorus content (0.79%) in seeds. Plants which were spaced at 15 cm × 5 cm spacing and applied with only 100 per cent RDF recorded significantly maximum phosphorus content (0.17%) in stems. ~~Whereas, p~~Plants which received treatment combination of 20 cm × 10 cm and application of 100 per cent RDF plus PGPR consortia recorded significantly maximum phosphorus content (0.20%) in roots. These interaction effects might be due to better plant growth at optimum spacing coupled with increased nutrients content and positive influence of PGPR consortia which might have enhanced the phosphorus mobilization thereby making it readily available to plants [31, 28]. Hence, PGPR applied plots along with FYM and inorganic fertilizers showed relatively maximum phosphorus content in different parts of hemp compared to control.

The potassium content in leaves and seeds of hemp differed significantly due to spacings, nutritions and interaction between them (Table 2). Significantly highest potassium content (1.05%) in leaves was noticed from plants spaced at 15 cm × 10 cm, whereas plants which were spaced at 10 cm × 10 cm recorded significantly highest potassium content (0.34%) in seeds. The widely spaced plants might have less competition for nutrients, water and light that resulted in better nutrient uptake by plants [30,34,24]. Among nutrition treatments, hemp plants nourished with 100 per cent RDF plus PGPR consortia recorded significantly maximum potassium content (0.91%) in leaves while, maximum potassium content (0.29%) in seeds was recorded in plants which received 75 per cent RDF plus PGPR consortia. The increased potassium content in leaves and seeds due to application of PGPR consortia along with FYM and inorganic fertilizers might be attributed to ability of biofertilizers in bringing the unavailable forms of potassium into soil solution and producing the bio-stimulants which helps in decomposition of soil organic matter [10]. This process helps to use potassium more easily by plants and further helps in increasing the accumulation [29]. ~~If interaction is considered,~~ Considering interactions, plants at 15 cm × 10 cm spacing and supplied with 75 per cent RDF plus PGPR consortia recorded significantly maximum potassium content in leaves (1.19%) whereas, treatment combination of 10 cm × 10 cm spacing and application of 75 per cent RDF with PGPR consortia recorded significantly maximum potassium content (0.72%) in seeds. This positive effect might be due to reduced competition among plants for natural resources at optimum spacing and positive effect of biofertilizers for easy

enhancement of available nutrients that helps to increase better nutrients uptake and increased potassium concentration [24].

Table 2. Primary nutrients content (%) in different parts of hemp as influenced by spacing and nutrition

Treatments	Total nitrogen (%)				Total phosphorus (%)				Total potassium (%)			
	Leaves	Stems	Seeds	Roots	Leaves	Stems	Seeds	Roots	Leaves	Stems	Seeds	Roots
S ₁	2.36	0.42	3.24	0.71	0.41	0.09	0.66	0.11	0.59	0.59	0.23	0.48
S ₂	2.05	0.39	2.80	0.69	0.31	0.09	0.56	0.09	0.43	0.61	0.34	0.67
S ₃	2.39	0.43	3.40	0.67	0.40	0.09	0.48	0.10	0.39	0.58	0.21	0.58
S ₄	2.51	0.31	3.53	0.74	0.34	0.07	0.47	0.10	1.05	0.60	0.18	0.67
S ₅	2.37	0.35	3.35	0.78	0.35	0.06	0.43	0.11	1.00	0.41	0.25	0.62
S ₆	2.39	0.33	3.09	0.83	0.34	0.09	0.48	0.11	0.95	0.44	0.10	0.54
S.Em ±	0.017	0.005	0.015	0.010	0.025	0.003	0.035	0.010	0.06	0.13	0.03	0.12
CD at 5%	0.05	0.012	0.04	0.03	NS	0.01	0.10	NS	0.16	NS	0.09	NS
N ₁	2.57	0.38	3.38	0.74	0.36	0.08	0.54	0.09	0.71	0.52	0.29	0.59
N ₂	2.52	0.38	3.30	0.68	0.42	0.07	0.50	0.13	0.91	0.52	0.27	0.62
N ₃	2.37	0.36	3.26	0.70	0.35	0.08	0.52	0.09	0.66	0.59	0.19	0.61
N ₄	1.92	0.35	3.00	0.83	0.29	0.09	0.49	0.10	0.66	0.51	0.12	0.55
S.Em ±	0.014	0.004	0.012	0.008	0.021	0.002	0.029	0.004	0.05	0.11	0.03	0.10
CD at 5%	0.04	0.01	0.04	0.03	0.06	0.01	NS	0.01	0.13	NS	0.08	NS
S ₁ N ₁	2.39	0.41	3.29	0.73	0.43	0.14	0.64	0.08	0.85	0.49	0.17	0.39
S ₁ N ₂	2.30	0.39	3.65	0.69	0.85	0.06	0.74	0.14	1.01	0.56	0.21	0.53
S ₁ N ₃	3.00	0.44	2.66	0.85	0.08	0.10	0.79	0.07	0.23	0.64	0.31	0.55
S ₁ N ₄	1.75	0.44	3.37	0.58	0.27	0.07	0.45	0.14	0.28	0.66	0.21	0.46
S ₂ N ₁	2.26	0.39	2.54	0.73	0.18	0.08	0.54	0.11	0.37	0.64	0.72	0.60
S ₂ N ₂	2.24	0.34	3.33	0.45	0.40	0.10	0.45	0.11	0.93	0.56	0.41	0.73
S ₂ N ₃	2.16	0.37	2.66	0.63	0.37	0.08	0.60	0.09	0.20	0.74	0.13	0.71
S ₂ N ₄	1.52	0.46	2.65	0.93	0.29	0.08	0.64	0.06	0.21	0.49	0.09	0.65
S ₃ N ₁	2.69	0.42	3.21	0.53	0.46	0.05	0.45	0.08	0.32	0.55	0.25	0.57
S ₃ N ₂	2.45	0.46	3.06	0.66	0.35	0.05	0.52	0.09	0.34	0.63	0.32	0.55
S ₃ N ₃	2.85	0.34	3.30	0.65	0.50	0.07	0.55	0.07	0.39	0.66	0.21	0.57
S ₃ N ₄	1.56	0.48	4.01	0.83	0.27	0.17	0.40	0.14	0.51	0.48	0.05	0.64
S ₄ N ₁	2.81	0.27	3.49	0.69	0.26	0.06	0.62	0.08	1.19	0.69	0.11	0.70
S ₄ N ₂	2.61	0.39	3.06	0.68	0.27	0.08	0.46	0.09	1.15	0.52	0.27	0.69
S ₄ N ₃	2.15	0.32	3.64	0.58	0.48	0.07	0.30	0.10	0.91	0.57	0.17	0.76
S ₄ N ₄	2.46	0.25	3.92	1.01	0.33	0.05	0.48	0.12	0.96	0.61	0.16	0.52
S ₅ N ₁	2.37	0.42	3.54	0.73	0.36	0.05	0.34	0.09	0.88	0.31	0.42	0.70
S ₅ N ₂	2.38	0.33	3.41	0.82	0.38	0.06	0.48	0.13	1.08	0.38	0.23	0.69
S ₅ N ₃	2.49	0.41	3.25	0.82	0.33	0.07	0.50	0.13	1.13	0.50	0.20	0.56
S ₅ N ₄	2.22	0.24	3.21	0.76	0.31	0.05	0.41	0.09	0.93	0.43	0.14	0.52
S ₆ N ₁	2.89	0.39	3.72	1.05	0.49	0.08	0.62	0.08	0.67	0.46	0.08	0.60
S ₆ N ₂	2.21	0.38	3.03	0.77	0.28	0.06	0.35	0.20	0.96	0.46	0.16	0.53
S ₆ N ₃	2.45	0.30	2.49	0.66	0.31	0.11	0.36	0.10	1.07	0.44	0.09	0.53
S ₆ N ₄	2.02	0.23	3.10	0.85	0.28	0.10	0.57	0.07	1.09	0.39	0.05	0.51
S.Em ±	0.034	0.010	0.029	0.020	0.051	0.005	0.071	0.010	0.11	0.26	0.06	0.24

CD at 5%	0.10	0.03	0.09	0.06	0.15	0.02	0.21	0.03	0.33	NS	0.18	NS
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3.3 Primary nutrients uptake

Maximum uptake of nitrogen by leaves ($198.25 \text{ kg ha}^{-1}$), seeds ($209.13 \text{ kg ha}^{-1}$) and whole plant ($450.32 \text{ kg ha}^{-1}$) were recorded when hemp plants were spaced at $15 \text{ cm} \times 10 \text{ cm}$ spacing (Figure 1). However, spacing of $10 \text{ cm} \times 10 \text{ cm}$ recorded maximum nitrogen uptake by stems (37.26 kg ha^{-1}) whereas, hemp plants at $10 \text{ cm} \times 5 \text{ cm}$ spacing recorded highest nitrogen uptake by roots (30.19 kg ha^{-1}). In case of nutrition treatments, hemp plants nourished with 75 per cent RDF plus PGPR consortia

documented-registered significantly maximum nitrogen uptake by leaves ($188.65 \text{ kg ha}^{-1}$) whereas, application of 125 per cent RDF plus PGPR consortia resulted in significantly maximum uptake of nitrogen by seeds ($202.76 \text{ kg ha}^{-1}$), stems (36.61 kg ha^{-1}) and whole plant ($440.33 \text{ kg ha}^{-1}$). However, least nitrogen uptake by all the hemp parts was noticed due to application of only inorganic fertilizers indicating the importance of biofertilizers in hemp cultivation. With respect to interactions, plants spaced at $15 \text{ cm} \times 10 \text{ cm}$ spacing and supplied with 100 per cent RDF through inorganic sources registered maximum nitrogen uptake by leaves ($235.48 \text{ kg ha}^{-1}$) whereas, treatment combination consisted of $10 \text{ cm} \times 5 \text{ cm}$ spacing and application of 75 per cent RDF plus PGPR consortia registered maximum uptake of nitrogen by roots (45.44 kg ha^{-1}). However, treatment combination of $20 \text{ cm} \times 5 \text{ cm}$ spacing and application of 75 per cent RDF plus PGPR consortia recorded highest nitrogen uptake by seeds ($257.62 \text{ kg ha}^{-1}$). Significantly maximum nitrogen uptake by stem (52.25 kg ha^{-1}) and whole plant ($531.20 \text{ kg ha}^{-1}$) was documented-registered by plants spaced at $10 \text{ cm} \times 5 \text{ cm}$ and provided with 125 per cent RDF plus PGPR consortia. The probable reason for increased uptake of nitrogen in medium denser treatments might be due to the fact that because there was possibility for the better shoot and root growth due to light interception which in turn increases root growth and better uptake of nitrogen from soil [26]. *Azospirillum brasilense* as component of PGPR might be responsible for increased nitrogen uptake compared to control [6]. Thus, dual application of

NS: Non significant

S₁: $10 \text{ cm} \times 5 \text{ cm}$

S₂: $10 \text{ cm} \times 10 \text{ cm}$

S₃: $15 \text{ cm} \times 5 \text{ cm}$

CD: Critical difference

S₄: $15 \text{ cm} \times 10 \text{ cm}$

S₅: $20 \text{ cm} \times 5 \text{ cm}$

S₆: $20 \text{ cm} \times 10 \text{ cm}$

N₁: 75% RDF + PGPR consortia

N₂: 100% RDF + PGPR consortia

N₃: 125% RDF + PGPR consortia

N₄: 100% RDF

Commented [A2]: Insert after table 2

inorganics and biofertilizers increased the growth, and nutrient assimilation due to their ability to fix atmospheric nitrogen and transform native soil nutrients such as P, Zn, Cu, Fe, and S from fixed to soluble form [29] and associative symbiotic nitrogen fixing bacteria *Azospirillum brasilense* produces adequate amounts of IAA and cytokinin that might have increased the surface area per unit root length and enhanced the root hair branching which facilitated the increased uptake of nutrients from the soil [13].

Phosphorus uptake by seeds, roots and total uptake by whole plants (39.08, 4.23 and 75.74 kg ha⁻¹, respectively) were highest when hemp plants were spaced at 10 cm × 5 cm spacing (Figure 2). However, maximum phosphorus uptake by stems (8.38 kg ha⁻¹) was noticed in plants spaced at 10 cm × 10 cm. The maximum phosphorus uptake by leaves (26.56 kg ha⁻¹) was found in plants which received 100 per cent RDF plus PGPR consortia. Whereas, Application of 125 per cent RDF plus PGPR consortia resulted in maximum phosphorus uptake by seeds (37.26 kg ha⁻¹), stems (8.25 kg ha⁻¹) and whole plants (72.58 kg ha⁻¹). Among interactions, combination of 10 cm × 5 cm spacing and application of 100 per cent RDF plus PGPR consortia resulted highest phosphorus uptake by leaves and whole plant (53.26 and 103.40 kg ha⁻¹, respectively). Whereas, treatment combination of 10 cm × 5 cm spacing and application of 125 per cent RDF plus PGPR consortia resulted in higher phosphorus uptake by seeds (63.75 kg ha⁻¹) and stems (11.88 kg ha⁻¹). However, plants from the plots which received treatment combination of 10 cm × 5 cm spacing and application of only 100 per cent RDF documented-registered maximum phosphorus uptake by roots (5.31 kg ha⁻¹). Phosphorus uptake was higher in plants spaced at closer spacing because phosphorus is highly immobile in soil and it is taken up by plant roots only by direct root contact mechanism. In closer spacing, plant roots come in contact with larger soil surfaces which resulted in higher uptake of phosphorus [26]. Combined application of inorganic fertilizers and biofertilizers shows increases phosphorous uptake when compared to the application of only chemical fertilizers might be due to the presence of phosphate solubilizing bacteria *Bacillus megatrium*

which is known to release organic acids which solubilizes unavailable phosphate compounds to readily available forms for plant uptake [6]. Further, *Pseudomonas fluorescens* have the ability to solubilize insoluble phosphate compounds in the soil and produce IAA and siderophores which are directly involved in nutrients uptake and plant growth [25].

The data on potassium uptake by different parts of hemp plant as influenced by spacing and nutrition indicated that the potassium uptake by leaves (82.83 kg ha^{-1}) and total uptake by whole plants ($159.13 \text{ kg ha}^{-1}$) was maximum with spacing of $15 \text{ cm} \times 10 \text{ cm}$. While, spacing of $10 \text{ cm} \times 10 \text{ cm}$ registered highest potassium uptake by seeds (18.46 kg ha^{-1}) and stems (61.49 kg ha^{-1}). Whereas, highest potassium uptake by roots (19.69 kg ha^{-1}) was observed in plants grown with at a spacing of $10 \text{ cm} \times 5 \text{ cm}$ (Figure 3). With regards to nutrition, application of 75 per cent RDF plus PGPR consortia registered maximum potassium uptake by leaves and roots (17.30 and 18.57 kg ha^{-1} , respectively). Whereas, soils supplied with 100 per cent RDF plus PGPR consortia recorded maximum potassium uptake by seeds (17.30 kg ha^{-1}) and total uptake by whole plant ($136.55 \text{ kg ha}^{-1}$). However, maximum potassium uptake by stems (60.41 kg ha^{-1}) was noticed in plants supplied with 125 per cent RDF plus PGPR consortia. Among interactions, treatment combination of $15 \text{ cm} \times 10 \text{ cm}$ spacing and application of 75 per cent RDF plus PGPR consortia recorded maximum uptake of potassium by leaves (98.45 kg ha^{-1}), Whereas, treatment combination of $10 \text{ cm} \times 10 \text{ cm}$ spacing and application of 75 per cent RDF plus PGPR consortia documented significantly highest potassium uptake by seeds (39.37 kg ha^{-1}). While, treatment combination of $10 \text{ cm} \times 10 \text{ cm}$ spacing and nutrition treatment of 125 per cent RDF plus PGPR consortia conglomerates recorded maximum uptake of potassium by stem (91.39 kg ha^{-1}). In the plots where plants were spaced at $15 \text{ cm} \times 10 \text{ cm}$ and applied with 125 per cent RDF plus PGPR consortia combination documented recorded significantly highest higher potassium uptake by roots (39.37 kg ha^{-1}) but maximum total potassium uptake by whole plant ($173.56 \text{ kg ha}^{-1}$) was registered from plots where plants were spaced at $15 \text{ cm} \times 10 \text{ cm}$ and applied with 100 per cent

RDF plus PGPR consortia. Better growth of the plants at optimum spacing resulted in ~~the~~ higher uptake of potassium from the soil [19]. Most probable reason for increased potassium uptake by plants which received PGPR consortia might be due to bacteria belonging to genera *Pseudomonas* and *Bacillus* which are able to solubilize the potassium bearing clay minerals such as biotite, muscovite, microcline and orthoclase thereby releasing potassium from them to soil solution in available form which can be easily assimilated by plants [13].

3.4 Chemical properties of soil

There was no significant difference in respect of soil pH, estimated after cropping due to various treatments imposed during experimentation (Table 3). However, the initial pH of soil was 6.57 and slightly increased after cropping and lies between neutral to slightly alkaline in reaction (6.79- 7.64). In general, organic sources tend to keep the soil pH in neutral range. However, a slight increase in soil pH could be ascribed to added organic matter to the soil [12]. Total soluble salt content of soils after the harvest of the crop was not significantly influenced by various spacing and nutrition treatments (Table 3). However, plants spaced at 15 cm × 10 cm and provided with 100 per cent RDF recorded significantly maximum electrical conductivity (0.25 dS m⁻¹) but within the limits of normal for optimum

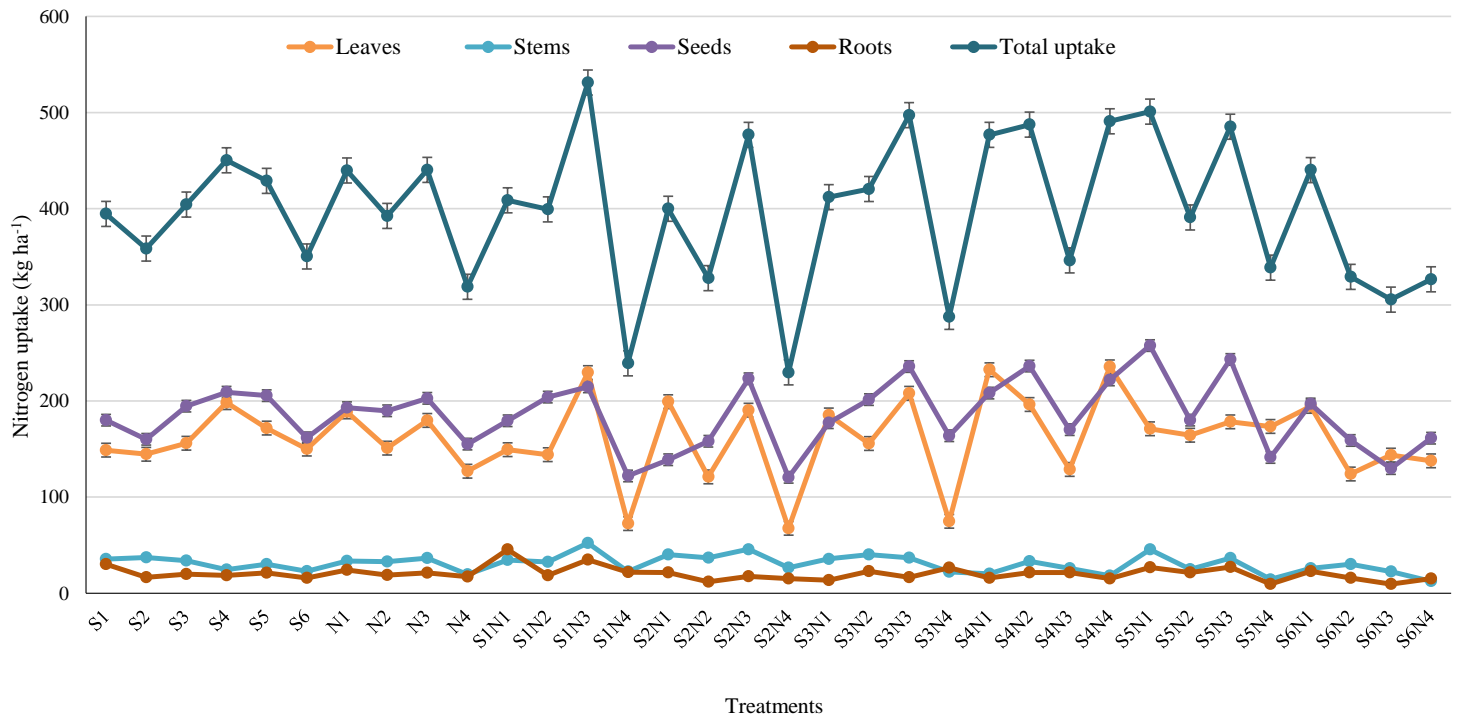


Figure 1. Nitrogen uptake (kg ha⁻¹) by different parts of hemp at harvest as influenced by spacing and nutrition treatments

Legend: S₁: 10 cm × 5 cm S₄: 15 cm × 10 cm N₁: 75% RDF + PGPR consortia
 S₂: 10 cm × 10 cm S₅: 20 cm × 5 cm N₂: 100% RDF + PGPR consortia
 S₃: 15 cm × 5 cm S₆: 20 cm × 10 cm N₃: 125% RDF + PGPR consortia

N₄: 100% RDF

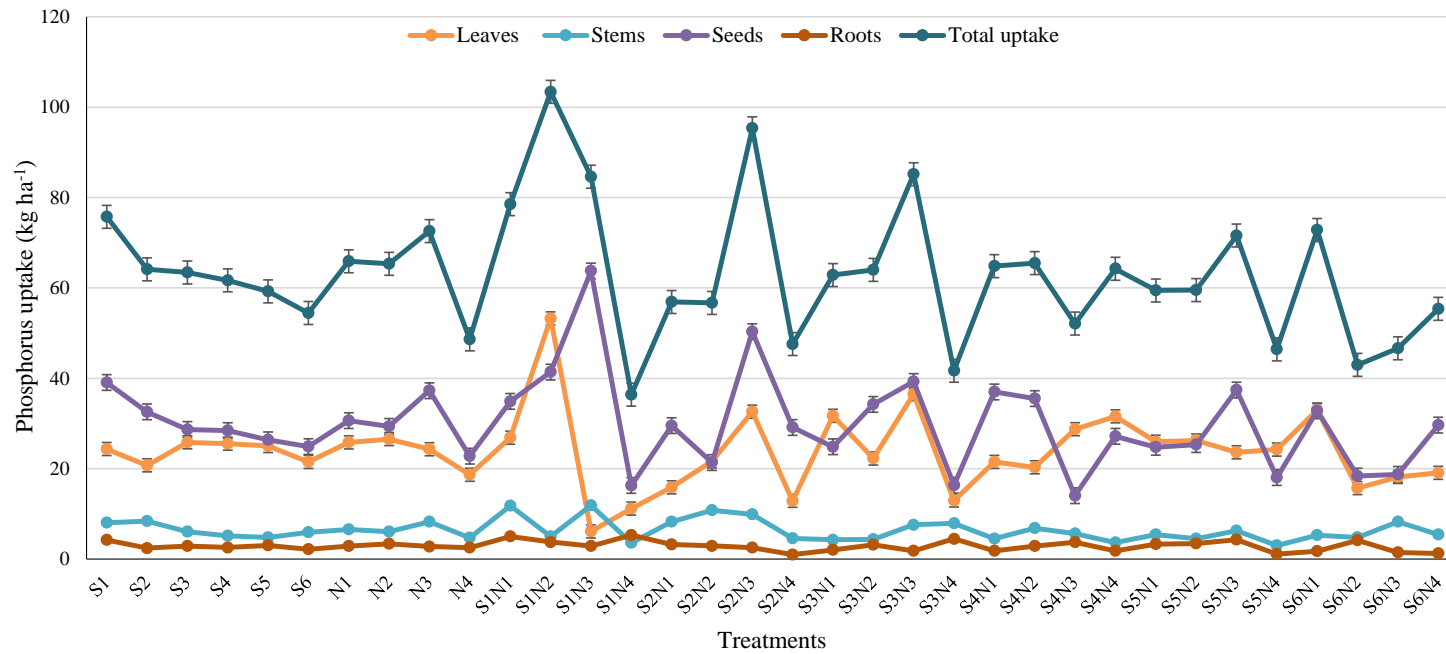
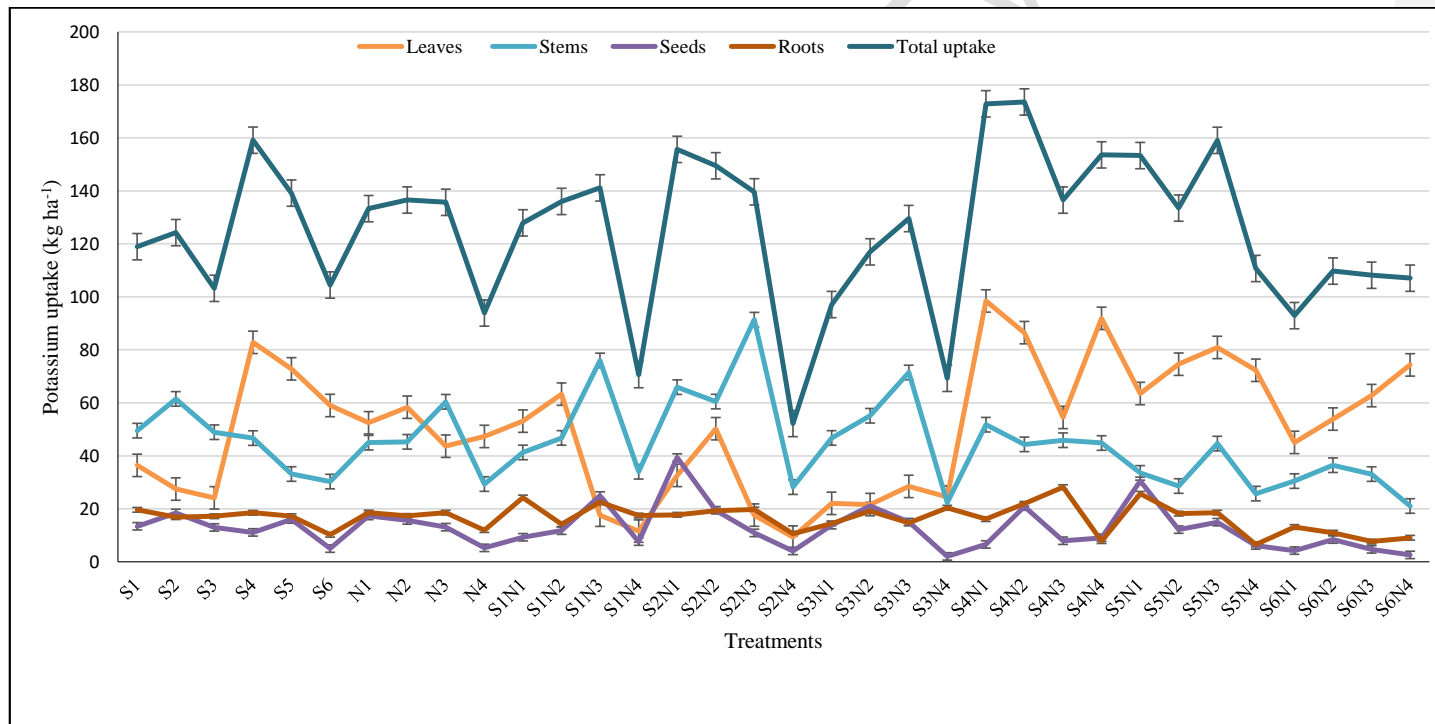


Figure 2. Phosphorus uptake (kg ha⁻¹) by different parts of hemp at harvest as influenced by spacing and nutrition treatments

Legend: S₁: 10 cm × 5 cm S₄: 15 cm × 10 cm N₁: 75% RDF + PGPR consortia
 S₂: 10 cm × 10 cm S₅: 20 cm × 5 cm N₂: 100% RDF + PGPR consortia
 S₃: 15 cm × 5 cm S₆: 20 cm × 10 cm N₃: 125% RDF + PGPR consortia
 N₄: 100% RDF



plant growth in all the treatment combinations tried. Spacing did not ~~had~~ have significant influence on organic carbon content of soils but application of 100 per cent RDF without PGPR consortia recorded significantly maximum organic carbon content (0.64%) ~~while,~~ Minimum organic carbon content (0.48%) of soil was noticed in plots which received 75 per cent RDF with PGPR consortia. Among interactions, hemp plants spaced at 10 cm × 5 cm ~~spacing~~ and provided with 100 per cent RDF registered maximum organic carbon content (0.72%) while, minimum was noticed in plots where plants spaced at 20 cm × 5 cm ~~spacing~~ and provided with 75 per cent RDF with PGPR consortia (Table 3). The maximum accumulation of organic matter in soils due to application of 100 per cent RDF without PGPR consortia might be attributed to less decomposition of added organic matter due to less microbial activities [2, 5, 15].

The available primary nutrients status of soil except potassium differed significantly due to different spacings, nutritions and interactions (Table 3). Significantly highest available nitrogen content (356.72 kg ha⁻¹) in the post-harvest soils of hemp was recorded in plots where plants were spaced at 10 cm × 10 cm while, lowest (294.00 kg ha⁻¹) was observed in soils where hemp plants ~~were~~ spaced at 20 cm × 5 cm s. Among nutrition treatments, application of 100 per cent RDF alone (control) resulted in significantly highest-higher available nitrogen content (376.32 kg ha⁻¹) over ~~the rest of the nutrition treatments tried~~ ~~which consisted of combined application of different levels of RDF and PGPR consortia (284.85 to 339.73 kg ha⁻¹)~~. Plants spaced at 10 cm × 5 cm spacing and applied with 100 per cent RDF ~~resulted in~~ maximum available nitrogen content (423.36 kg ha⁻¹) of soil while, least (250.88 kg ha⁻¹) was noticed in the plots where plants were spaced at 20 cm × 5 cm and supplied with 75 per cent RDF with PGPR consortia. ~~In general,~~ ~~The~~ maximum available nitrogen content in post-harvest soils of hemp was observed in plots where plants were planted at closer spacing [8] and supplied with only inorganic fertilizers. This might be due to the lack of biofertilizers that might have decreased the fertilizers use efficiency thereby ~~increased~~ increasing the available nitrogen in soils [1].

Significantly highest-higher available phosphorus content of 25.34 kg P₂O₅ ha⁻¹ was recorded in plots where plants were spaced at 15 cm × 10 cm while, lowest (21.47 kg P₂O₅ ha⁻¹) was observed in soils where hemp plants spaced at 20 cm × 5 cm spacing. Significantly highest available phosphorus content of 27.06 kg P₂O₅ ha⁻¹ was recorded in soils applied with 125 per cent RDF plus PGPR consortia over all other nutrition treatments tried. ~~Whereas,~~ Significantly least available phosphorus content of 19.40 kg P₂O₅ ha⁻¹ was recorded in soils supplied with 100 per cent RDF through inorganic sources. Among interactions, plots ~~in~~ ~~with plants~~ ~~which hemp plants~~ ~~were~~ spaced at 10 cm × 5 cm and applied with 125 per cent RDF plus PGPR consortia documented higher available phosphorus content of 29.75 kg P₂O₅ ha⁻¹ in soil. More availability of nutrients and lesser uptake of phosphorus by the crop at high-density planting might have resulted in high availability of phosphorus in post-

harvest soils [26, 6]. When soluble inorganic phosphatic fertilizers are added to soil, they get fixed as insoluble calcium phosphates in neutral, calcareous and saline soils and as insoluble iron and aluminium phosphates in acid soils but treatments having PGPR consortia along with inorganic fertilizers had higher availability of phosphorus due to continuous mineralization and conversion of insoluble phosphates to soluble forms. Kohler [17] reported that secretion of phosphatase enzyme by PSB and *Bacillus megatherium* is a mode of facilitating the conversion of insoluble forms of phosphorus to soluble forms and thus enhances phosphorus uptake by plants. Thus, increased availability of phosphorus in the soil increases phosphorus absorption as well as phosphorus accumulation in plant tissues [19, 8]. The available potassium status of soil remained insignificant among treatments because experiment was conducted on uniform nature of soil and potassium fixation in soils is mainly influenced by the kind and amount of secondary clay minerals present and its availability is not influenced by soil pH. However, the available potassium content of all soils was medium in rating and the values ranged from 261.27 to 314.50 kg K₂O ha⁻¹.

Table 3. Chemical properties of soil as influenced by spacing and nutrition of hemp

Characters	pH	EC (dS m ⁻¹)	OC (%)	Avail. N (kg ha ⁻¹)	Avail. P ₂ O ₅ (kg ha ⁻¹)	Avail. K ₂ O (kg ha ⁻¹)
S ₁	7.14	0.19	0.58	341.04	24.18	286.51
S ₂	7.10	0.19	0.61	356.72	23.31	277.57
S ₃	7.04	0.16	0.56	329.28	21.93	283.00
S ₄	7.30	0.17	0.53	313.60	25.34	272.71
S ₅	7.06	0.19	0.50	294.00	21.47	275.08
S ₆	7.06	0.18	0.56	329.28	21.66	289.28
S.Em ±	0.11	0.01	0.03	14.42	0.84	14.00
CD at 5%	NS	NS	NS	42.20	2.47	NS
N ₁	7.14	0.19	0.48	284.85	22.97	268.67
N ₂	7.03	0.18	0.52	308.37	22.49	283.91
N ₃	7.17	0.18	0.58	339.73	27.06	287.24
N ₄	7.13	0.18	0.64	376.32	19.40	282.94
S.Em ±	0.09	0.01	0.02	11.78	0.68	11.43
CD at 5%	NS	NS	0.06	34.66	2.01	NS
S ₁ N ₁	7.04	0.15	0.48	282.24	22.57	270.64
S ₁ N ₂	6.99	0.24	0.51	297.92	27.62	290.86
S ₁ N ₃	7.53	0.20	0.61	360.64	29.75	295.10
S ₁ N ₄	7.02	0.18	0.72	423.36	16.76	289.42
S ₂ N ₁	7.22	0.23	0.56	329.28	22.70	261.27
S ₂ N ₂	6.88	0.15	0.56	329.28	25.78	275.83
S ₂ N ₃	7.37	0.24	0.64	376.32	28.73	283.33
S ₂ N ₄	6.94	0.16	0.67	392.00	16.03	289.83
S ₃ N ₁	6.90	0.20	0.45	266.56	13.21	266.89
S ₃ N ₂	7.09	0.21	0.51	297.92	21.16	285.76
S ₃ N ₃	7.05	0.16	0.61	360.64	24.88	283.54
S ₃ N ₄	7.14	0.08	0.67	392.00	28.46	295.81
S ₄ N ₁	7.15	0.18	0.48	282.24	28.93	263.94
S ₄ N ₂	7.01	0.17	0.53	313.60	24.60	264.92
S ₄ N ₃	7.39	0.09	0.53	313.60	24.49	295.98
S ₄ N ₄	7.64	0.25	0.59	344.96	23.34	266.03
S ₅ N ₁	7.36	0.22	0.43	250.88	25.15	269.70
S ₅ N ₂	6.92	0.13	0.48	282.24	16.74	271.63
S ₅ N ₃	6.79	0.20	0.51	297.92	27.83	287.87

S ₅ N ₄	7.18	0.22	0.59	344.96	16.16	271.11
S ₆ N ₁	7.19	0.16	0.51	297.92	25.27	279.57
S ₆ N ₂	7.32	0.18	0.56	329.28	19.04	314.50
S ₆ N ₃	6.88	0.18	0.56	329.28	26.67	277.65
S ₆ N ₄	6.85	0.21	0.61	360.64	15.65	285.41
S.Em ±	0.22	0.02	0.05	28.84	1.68	28.01
CD at 5%	NS	0.07	0.14	84.40	4.93	NS

4. CONCLUSION

From the insights of above findings, it can be inferred that spacing plays an important role in determining yield per unit area and nutrition can be considered as advantageous means for enhancing growth and yield. Spacing of 15 cm × 10 cm is found to be optimum for getting enhanced yield and maximum uptake of nutrients by different parts of hemp. Application of 10 t FYM ha⁻¹ along with 125 per cent of RDF (250:125:250 kg NPK ha⁻¹) plus PGPR consortia provides an opportunity to explore maximum yield potential of hemp crop.

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NS: Non-significant
S₁: 10 cm × 5 cm
S₂: 10 cm × 10 cm
S₃: 15 cm × 5 cm

CD: Critical difference
S₄: 15 cm × 10 cm
S₅: 20 cm × 5 cm
S₆: 20 cm × 10 cm

N₁: 75% RDF + PGPR consortia
N₂: 100% RDF + PGPR consortia
N₃: 125% RDF + PGPR consortia
N₄: 100% RDF

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Abbreviations: DAT: Days After Transplanting, PGPR: Plant growth promoting rhizobacteria, RDF: Recommended Dose of Fertilizers

UNDER PEER REVIEW

