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Osmopriming Combined with Boron-Tolerant Bacteria (*Bacillus* sp. MN54) Improved the Productivity of Desi Chickpea under Rainfed and Irrigated Conditions

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Abstract: Chickpeas are rich source of protein and predominantly grown in boron (B)-deficient sandy-loam soils in Pakistan. Boron-tolerant bacteria (BTB) could tolerate higher B levels in soil and increase B availability to the plants. Field trials were conducted under irrigated (district Layyah) and rainfed (district Chakwal) conditions to evaluate the interactive effects of pre-optimized B application methods and BTB (*Bacillus* sp. MN54) on the nodule's population, grain quality, productivity, and grain-B concentration in desi chickpea during 2019–2020 and 2020–2021. Boron was applied as soil application (1 kg B ha^{-1}), foliar application (0.025% B), osmopriming (0.001% B), and seed coating (1.5 g B kg^{-1} seed) with or without BTB inoculation. Untreated seeds receiving no B through any of the methods were regarded as control. The individual and interactive effects (up to three-way interaction of location × BTB inoculation × B application methods) of year, location, B application methods and BTB inoculation significantly altered the growth and yield-related traits of desi chickpea. The four-way interaction of year × location × BTB inoculation × B application methods was non-significant for all recorded growth and yield-related traits. Regarding individual effects, the higher values of growth and yield-related traits were noted for 2020–2021, rainfed location, BTB inoculation and B application through seed priming. Similarly, in two-way interactions 2020–2021 with rainfed location and BTB inoculation, rainfed location with BTB inoculation and osmopriming and osmopriming with BTB inoculation recorded higher values of the growth and yield-related traits. Osmopriming combined with BTB inoculation significantly improved dry matter accumulation and leaf area index in both locations. Boron application through all the methods significantly improved grain quality, yield grain B concentration. The highest grain and biological yields, and nodules' population were recorded with osmopriming followed by soil application of B combined with BTB inoculation. The highest plant B concentration (75.05%) was recorded with foliar application of B followed by osmopriming (68.73%) combined with BTB inoculation. Moreover, the highest economic returns (USD 2068.5 ha^{-1}) and benefit–cost ratio (3.7%) were recorded with osmopriming + BTB inoculation in 2020–2021 under rainfed conditions. Overall, B application through osmopriming and soil application combined with BTB inoculation could be used to increase productivity and profitability of desi chickpea, whereas foliar application is a better method to enhance grain and plant B concentration.

Keywords: osmopriming; grain yield; grain B concentration; boron-tolerant bacteria

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1. Introduction

Balanced nutrition is essential for optimum growth and quality seed production in crop plants. Micro- and macronutrients are equally important for better crop growth and productivity [1]. The farmers seldom use recommended quantities of micronutrients and mostly focus on primitive methods of macronutrients' application which result in nutritional imbalance [2]. Micronutrients are less mobile within plants; therefore, must be supplied for better growth and productivity [3,4]. Boron is a micronutrient and its intake in daily diet is permissible up to 20 mg day^{-1} in adults and 1.5–3 mg day^{-1} in children under the age of three years. Boron controls many diseases, including memory loss, and low testosterone level.

Boron is required in minute quantity; thus, its toxicity and deficiency both are detrimental for plants. Soils are mostly deficient in B as compared to other micronutrients, as B-deficiency is observed in ~80 countries across the globe, including Pakistan. It has been observed that 65% of Pakistani soils are B-deficient on which 132 plant species are being cultivated [5–7].

Like other plant species, B is important for chickpea (*Cicer arietinum* L.) as it plays significant role in cell wall synthesis, cell division, pod formation, seed setting, nodulation and improving seed quality [8–10]. Insufficient B supply restricts seedling growth, diminishes fruit quality, decreases number of grains per spike and increases anthesis which alters seed setting [11,12]. Excessive B supply reduces chlorophyll contents and chloroplast in leaves, and results in water loss during stomatal limitations, ultimately lowering photosynthetic capacity [13–16]. Chickpea is a leguminous crop and Pakistan ranks 4th in term of production in the world. Chickpea is cultivated on both irrigated and rainfed lands in Pakistan [17]. Two types of chickpeas (i.e., desi and kabuli) are cultivated in the world. Desi type has small size and angular shape, which is cultivated on ~80–85% of the area in the Indo-Pak subcontinent [18].

Biofortification is the process of improving micronutrients' density in crop plants through improved agronomic practices, conventional breeding, and modern biotechnological approaches. Agronomic biofortification is gaining importance, in which nutrient status of crop plants can be enhanced by using different application methods such as seed treatment (priming and coating), foliar and soil application [19]. Plants can easily uptake nutrients through soil application, and it is one of the primitive and beneficial methods of biofortification. It has been observed that soil-applied B along with *Bacillus* sp. strain MN54 considerably increased roots' growth, nodulation, grain production and nutritional quality of chickpea [20]. Foliar feeding is the direct application of nutrients on the plant canopy, especially leaves, to improve grain yield and nutritional value of crop [21,22]. It has been reported foliar spray of B at booting stage produced higher grain yield in several cereal crops [12,23].

Seed coating is the most economical method of nutrient application directly to seeds which involves adhering the required nutrients to the seed surface [24,25]. Boron seed coating in chickpea improved grain yield by 25%, whereas B seed coating in tandem with inoculation of boron-tolerant bacteria (BTB) MN54 increased grain yield by 37% compared to untreated seeds [26]. Seed priming is a controlled hydration technique that involves soaking of seeds in liquid osmotica for a specific time and then seeds are re-dried to their original weight [27–29]. Combined application of B seed priming and bacterial inoculation has been found helpful to improve early stand establishment, seedling emergence, grains' productivity and grain biofortification of different crops [30–32].

Microbial inoculation has been extensively used to improve the mobility of micronutrients in the soil and their availability to the plants, which correspondingly avoid the excessive use of chemical fertilizers [33]. The BTB has the ability to survive under harsh conditions, tolerate B-toxicity and deficiency, which promote crop growth and grain production [34]. Recent studies clearly showed that combined application of *Bacillus* spp. MN-54 and B through seed coating [26], soil application [20] and seed priming [32] improved B uptake and productivity of chickpea. The BTB enables plants to survive under higher B level by controlling its dynamics. Under harsh environmental and nutritional conditions, survival rate of normal bacteria is very low; however, BTB could survive under these harsh conditions and lower toxic effects of B.

Several studies have been conducted to evaluate the positive effects of B fertilization along with bacterial strains' inoculation on different crops; however, information regarding combined application of pre-optimized B levels for different application methods and BTB strains on the productivity of desi chickpea has not reported yet. We optimized the B application levels for different methods in pot experiments in earlier studies and then tested these optimized levels under field conditions in the current study. Thus, the main objectives of this study were to assess the most suitable B application method along with BTB inoculation to improve growth, grain productivity, quality and biofortification of desi chickpea under rainfed and irrigated conditions. It was hypothesized that combined application of BTB and B through different methods would increase the growth, nodulation, grain quality, productivity and biofortification of chickpea. It was further hypothesized that osmopriming combined with BTB inoculation will improve productivity, whereas foliar application of B coupled with BTB inoculation would improve plant and grain B concentration.

2. Materials and Methods

2.1. Experimental Site

Field experiments were conducted at irrigated (Bahauddin Zakariya University, Bahadur Sub Campus, Layyah, 30°57′36.0″ N, 70°55′48.0″ E) and rainfed (Research Farm Chakwal, 32°55′48.0″ N, 72°51′36.0″ E) conditions during 2019–2020 and 2020–2021. The B levels used in different methods in the current study were selected based on earlier studies [20,26,32]. Before sowing, the soils of the experimental sites were analyzed for physical and chemical properties which are given in Table 1.

Table 1. Soil physical and chemical properties of experiment locations prior to the initiation of experiments.

Soil Properties	2019–2020		2020–2021	
	Rainfed Area	Irrigated Area	Rainfed Area	Irrigated Area
Soil texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam
EC	1.12 dS m^{-1}	3.52 dS m^{-1}	1.14 dS m^{-1}	3.48 dS m^{-1}
pH	7.35	8.00	7.50	8.20
Saturation	36.00%	30.00%	36.00%	32.00%
Organic matter	0.41%	0.55%	0.35%	0.53%
Available phosphorous	3.23 mg kg^{-1}	5.43 mg kg^{-1}	3.32 mg kg^{-1}	4.92 mg kg^{-1}
Available potassium	78 mg kg^{-1}	132 mg kg^{-1}	74 mg kg^{-1}	134 mg kg^{-1}
Available nitrogen	0.032%	0.053%	0.041%	0.049%
Available boron	0.32 mg kg^{-1}	0.45 mg kg^{-1}	0.35 mg kg^{-1}	0.42 mg kg^{-1}

2.2. Planting Material and Treatment Details

The best performing desi chickpea variety 'Punjab-2008′ based on an earlier optimization study was used in the current study due to its better performance. All possible combinations of two inoculation levels (control and inoculated) and B application methods (seed priming, seed coating, foliar and soil application) were tested under two locations (irrigated and rain-fed). Seeds were coated with 1.5 g B kg^{-1} by using Arabic gum as the adhering agent.

For soil application, 1 kg B ha^{-1} was applied in each plot through side dressing. For osmopriming, seeds were soaked in aerated B solution (0.001% B), while seeds were soaked in distilled water only for hydropriming. In foliar application, 0.025% B solution was sprayed 55 days after sowing at N-node stage (unfolded leaf and flat leaflets before flowering) on plant leaves. Water spray with distilled water, untreated dry seeds and hydro-priming were taken as control to compare with B application treatments. Boric acid (CAS No. 10043-35-3, a product of Merck, Darmstadt, Germany) was used as B source. The seeds in all treatments were either B-inoculated or non-inoculated and sown. The experiment was laid out according to randomized complete block design with factorial arrangements. All treatments had three replications. The row-to-row and plant-to-plant distance was 45 and 10 cm, respectively. The net plot size was 5 × 3 m.

Optical density bacterial strain *Bacillus* sp. MN54 (Accession no. KT375574) of inoculum was adjusted to 109 cfu per mL before seed inoculation. This strain has already been used for enhancing growth and production of different crops [35–37].

2.3. Crop Husbandry

The seeds were sown on well-prepared seedbeds at both locations. Before sowing, pre-soaking irrigation of 10 cm was applied to the irrigated experiment. When soil reached a workable condition, two cultivations followed by planking were completed. Sowing was undertaken on 24th and 26th of October during 2019, and 20th and 23rd October during 2020 in irrigated and rainfed locations, respectively. Sowing was undertaken with the help of a hand drill by using a seed rate of 80 kg ha^{-1}. Before sowing 40 kg ha^{-1} nitrogen and 80 kg ha^{-1} phosphorous was applied using urea and tipple super phosphate to fulfill nutrient requirements. In the irrigated location, four irrigations were applied to avoid moisture stress, whereas rainwater was the only moisture source under rainfed conditions. Weeds were manually controlled at both locations. Mature crop was harvested on 18 April and 4 May 2020, and 1 and 18 April 2021 in irrigated and rainfed locations, respectively.

2.4. Observations

2.4.1. Nodule Population

Three plants were uprooted randomly form each treatment before flowering and the number of nodules was counted and averaged to record the nodule population.

2.4.2. Allometric Traits

Chlorophyll index was measured by taking the Soil Plant Analysis Development (SPAD) value (SPAD-502, Minolta, Konica Minolta Inc., Osaka, Japan) of five randomly selected leaves of different plants from each treatment.

Three plants from each treatment were taken and their leaves were separated from the stem. The area of the detached leaves was determined by leaf area meter (DT Area Meter, Model MK2, Delta T Devices, Cambridge, UK). Afterwards, leaf area index (LAI) was calculated by using following formula given by Watson [38].

$$\text{LAI} = \frac{\text{Leaf area}}{\text{Ground area}} \tag{1}$$

Crop growth rate (CGR) was determined by harvesting the plants from an area of 1 m^2. Sampling was completed from 35 to 95 DAS with an interval of 20 days. All the samples were weighed to take fresh weight and then sun dried in open air. Furthermore, sun-dried samples were placed in a hot air oven for 48 h at 70 °C for drying. Oven-dried samples were weighed and CGR was calculated by using the following formula given by Hunt [39].

$$\text{CGR} = \frac{\text{W2} - \text{W1}}{\text{t2} - \text{t1}} \tag{2}$$

In the equation, W_2 = dry weight per unit land area (g m^{-2}) at second harvest, W_1 = dry weight per unit land area (g m^{-2}) at first harvest, t_2 = time corresponding to second harvest and t_1 = time corresponding to first harvest.

2.4.3. Yield and Related Traits

Number of pods per plant were counted by picking pods from 10 randomly selected plants from each plot. Number of grains per pod were counted from manually threshed 20 pods from each plot and averaged. Number of grains per plant was calculated by using the formula given by Hussain et al. [26].

$$Number\ of\ grains\ per\ plant = Number\ of\ pods\ per\ plant \times Number\ of\ grains\ per\ pod$$

For estimating 1000-grain weight, three samples of 100 grains from each plot were taken, weighed and multiplied by 10 to record 1000-grain weight. Plants were harvested from an area of 1 m^2 from each experimental unit, sun dried and weighed to get biological yield, followed by threshing to account for grain yield. Then, biological yield and grain yield of 1 m^2 were converted into hectares by using the unitary method. Harvest index was calculated as:

$$\text{Harvest index} = \frac{\text{Grain yield}}{\text{Biological yield}} \tag{3}$$

2.4.4. Grain Boron Analysis

Grain and leaf samples were oven dried at 70 °C for B analysis. After drying, 1 g plant and grain sample from each treatment was taken in a porcelain crucible and incinerated in a muffle furnace at 550 °C for 6 h [40]. Extraction of samples was completed by adding 10 mL of 0.36 N H_2SO_4 for 1 h. Extracted samples were then filtered with Whatman no. 1 filter paper and transferred to 50 mL transparent plastic bottles and the volume was raised to 50 mL by adding distilled water. Buffer solution was prepared by adding 250 mL ammonium acetate, 15 mL Ethylenediamine tetraacetic acid (EDTA) and 125 mL acetic acid gently into 400 mL distilled water. The azomethine solution was formed by adding 0.45 g azomethine-H and 1 g L-ascorbic acid into 100 mL of distilled water. Afterwards, 1 mL solution was taken from filtered extracted solution and mixed with 2 mL of each buffer solution and azomethine-H solution. Prepared samples were kept at room temperature for 45 min to develop color. After that, samples were analyzed for grain and plant B concentrations by using a spectrophotometer (double beam product of Bristol Myers Squibb, Madrid, Spain) at 420 nm wavelength.

2.5. Statistical and Economic Analysis

The recorded data were tested for normality and the variables with non-normal distribution were normalized using Arcsine transformation technique to meet the normality assumption of analysis of variance (ANOVA). A four-way ANOVA (year × B application methods × BTB inoculation × location) was used to test the significance in the dataset. Least significant difference at 95% probability level was used to compare the means where ANOVA denoted significant differences [41]. Economic analysis was undertaken to test the economic efficiency of desi chickpea grown with various B application methods combined with BTB inoculation. For economic analysis, production costs (cost required for land rent, seedbed preparation, sowing and harvesting of crop, and purchase of inputs such as seed, fertilizer pesticides and irrigation) were computed. Net income was attained by excluding all expenses from the gross income. Gross income was divided production cost to compute benefit–cost ratio (BCR) [42]. The significant interactions were presented and interpreted in the manuscript.

3. Results

3.1. Nodules' Population

Nodules' population was significantly altered by individual effects of year, locations, BTB inoculation and B application methods. The significant two-way interactions were year × location, year × BTB inoculation and location × BTB inoculation, while the remaining possible interactions were non-significant (Table 2).

Regarding individual effects, 2020–2021, rainfed location, BTB inoculation and osmopriming recorded higher root nodulation compared to the remaining individual effects of the study (Table 3). Rainfed location and BTB inoculation interaction with 2020–2021 and rainfed location by BTB inoculation interaction recorded higher root nodulation compared to the other possible interactions (Table 4). The three-way and four-way interactions remained non-significant.

Table 2. Analysis of variance (*p* values) of individual and interactive effects of year, location, BTB (*Bacillus* sp. MN54) inoculation and boron nutrition for growth and yield-related traits of desi chickpea.

Variables	Y	S	B	I	Y × S	Y × B	Y × I	S × B	S × I	B × I	Y × S × B	Y × S × I	Y × B × I	S × B × I	Y × S × B × I
Nodule population	0.0000 *	0.0009 *	0.0000 *	0.0000 *	0.0024 *	0.0740 NS	0.0248 *	0.1118 NS	0.0358 *	0.1770 NS	0.5980 NS	0.2101 NS	0.5498 NS	0.9538 NS	0.8909 NS
Number of pods per plant	0.0000 *	0.0000 *	0.0000 *	0.0000 *	0.0084 *	0.0448 *	0.0040 *	0.0000 *	0.0019 *	0.0001 *	0.0448 *	0.9040 NS	0.9917 NS	0.0000 *	0.9917 NS
Number of grains per pod	0.0000 *	0.0000 *	0.0000 *	0.0000 *	0.4902 NS	0.6013 NS	0.9738 NS	0.6926 NS	0.5763 NS	0.1286 NS	0.9422 NS	0.9214 NS	0.9499 NS	0.9388 NS	0.9769 NS
Number of grains per plant	0.0000 *	0.0000 *	0.0000 *	0.0000 *	0.0000 *	0.1407 NS	0.7561 NS	0.0001 *	0.4804 NS	0.0159 *	0.0001 *	0.2725 NS	0.9262 NS	0.0000 *	0.7403 NS
1000-grain weight (g)	0.0000 *	0.0000 *	0.0000 *	0.0000 *	0.0000 *	0.8217 NS	0.4896 NS	0.0000 *	0.2835 NS	0.0218 *	0.8217 NS	0.4896 NS	0.8217 NS	0.0000 *	0.8217 NS
Grain yield (t ha^{-1})	0.0000 *	0.0000 *	0.0000 *	0.0000 *	0.0000 *	0.1392 NS	0.0124 *	0.0000 *	0.0911 NS	0.0006 *	0.1392 NS	0.9762 NS	0.1392 NS	0.0000 *	0.1392 NS
Biological yield (t ha^{-1})	0.0000 *	0.0000 *	0.0000 *	0.0000 *	0.0000 *	0.8715 NS	0.9936 NS	0.0000 *	0.0000 *	0.0000 *	0.8715 NS	0.9936 NS	0.9629 NS	0.0000 *	0.9629 NS
Harvest index (%)	0.2466 NS	0.0000 *	0.0001 *	0.0035 *	0.0000 *	0.2601 NS	0.2476 NS	0.0000 *	0.0015 *	0.0000 *	0.6734 NS	0.8382 NS	0.9735 NS	0.0000 *	0.4769 NS
Grain B concentration (mg)	0.0000 *	0.0000 *	0.0000 *	0.0000 *	0.0000 *	0.0399 *	0.0003 *	0.1302 NS	0.0091 *	0.2548 NS	0.6130 NS	0.0801 NS	0.2105 NS	0.0001 *	0.064 NS
Plant B concentration (mg)	0.0000 *	0.0000 *	0.0000 *	0.0000 *	0.0000 *	0.0615 NS	0.0011 *	0.0024 *	0.0166 *	0.3670 NS	0.0003 *	0.1120 NS	0.0320 *	0.7781 NS	0.1054 NS

Here: Y = year; S = locations (i.e., irrigated or rainfed); B = boron application methods; I = BTB (*Bacillus* sp. MN54) inoculation; * indicates that the relevant individual or interactive effect is significant for the respective trait, where NS indicates that the individual or interactive effect is non-significant for the respective trait.

Table 3. The impact of year, location, BTB (*Bacillus* sp. MN54) inoculation and boron nutrition on growth and yield-related traits of desi chickpea.

	Nodule Population	Number of Pods per Plant	Number of Grains per Pod	Number of Grains per Plant	1000-Grain Weight (g)	Grain Yield (t ha^{-1})	Biological Yield (t ha^{-1})	Harvest Index (%)	Grain B Concentration (mg)	Plant B Concentration (mg)
Years										
Y_1	20.31 b	31.94 b	2.28 b	75.48 b	227.63 b	2.41 b	5.27 b	-	40.24 b	43.11 b
Y_2	25.93 a	34.31 a	2.49 a	95.84 a	236.55 a	2.60 a	5.71 a	-	48.39 a	50.30 a
LSD 0.05	1.53	0.71	0.07	2.55	3.45	0.11	0.06	-	1.33	1.62
Locations										
S_1	24.44 a	34.36 a	2.46 a	94.94 a	236.09 a	2.46 b	6.33 a	39.17 b	42.68 b	48.82 a
S_2	21.80 b	31.89 b	2.31 b	76.38 b	228.09 b	2.55 a	4.64 b	54.88 a	45.95 a	44.60 b
LSD 0.05	1.54	0.75	0.10	2.87	4.21	0.07	0.12	0.54	1.76	2.13
Bacterial inoculation										
I_1	26.21 a	37.84 a	2.54 a	101.29 a	239.24 a	2.66 a	5.80 a	47.43 a	47.81 a	49.93 a
I_2	20.02 b	28.41 b	2.23 b	70.03 b	224.94 b	2.35 b	5.18 b	46.62 b	40.82 b	43.48 b
LSD 0.05	1.56	0.77	0.08	3.01	4.33	0.12	0.17	0.48	2.45	2.93
Boron application methods										
B_1	15.38 e	23.85 f	1.90 e	53.19 f	216.28 g	2.21 f	4.73 e	47.39 a	20.37 e	22.37 e
B_2	15.71 e	26.79 e	2.07 d	62.92 e	223.04 e	2.34 e	4.99 d	47.95 a	19.95 e	23.08 e
B_3	23.25 c	33.83 c	2.51 b	88.15 c	235.33 c	2.57 c	5.57 c	47.45 a	69.80 a	71.08 a
B_4	29.46 b	38.19 b	2.63 b	103.48 b	243.89 b	2.71 b	5.96 b	47.05 ab	56.56 c	59.00 c
B_5	18.92 d	29.94 d	2.25 c	73.81 d	220.11 f	2.35 e	5.03 d	47.52 a	23.47 d	26.88 d
B_6	34.54 a	45.51 a	2.79 a	129.78 a	255.72 a	2.88 a	6.50 a	46.25 bc	63.49 b	65.52 b
B_7	24.58 c	33.77 c	2.55 b	88.29 c	230.26 d	2.48 d	5.62 c	45.59 c	56.56 c	59.00 c
LSD 0.05	2.87	1.32	0.13	4.77	3.90	0.06	0.11	1.01	2.49	3.04

Here: Y_1 = 2019–2020; Y_2 = 2020–2021; S_1 = rainfed; S_2 = irrigated; I_1 = BTB inoculation; I_2 = no BTB inoculation; B_1 = control (0.00); B_2 = water spray; B_3 = foliar spray at 0.05% B; B_4 = soil application; B_5 = hydropriming; B_6 = osmopriming 0.001% B; and B_7 = seed coating 1.5/kg seed. LSD = least significant difference. Any two means sharing one letter in common within a column are statistically similar at 95% probability level. - = the relevant parameter was not significantly altered by the respective explanatory variable.

Table 4. The impact of year by location, year by BTB (*Bacillus* sp. MN54) inoculation and location by BTB (*Bacillus* sp. MN54) inoculation interaction on growth and yield-related traits of desi chickpea.

	Nodule Population	Number of Pods per Plant	Number of Grains per Plant	1000-Grain Weight (g)	Grain Yield (t ha^{-1})	Biological Yield (t ha^{-1})	Harvest Index (%)	Grain B Concentration (mg)	Plant B Concentration (mg)
					Year × Location				
Y_1S_1	20.43 c	32.86 b	80.62 b	230.09 b	2.39 d	6.01 b	40.15 c	40.36 c	43.21 c
Y_1S_2	20.19 c	31.02 c	70.33 c	225.18 c	2.42 c	4.53 d	53.59 b	40.12 c	43.00 c
Y_2S_1	28.45 a	35.86 a	109.26 a	242.09 a	2.53 b	6.66 a	38.20 d	44.99 b	54.42 a
Y_2S_2	23.41 b	32.77 b	82.42 b	231.00 b	2.67 a	4.76 c	56.17 a	51.78 a	46.19 b
LSD 0.05	2.16	1.04	3.61	4.44	0.02	0.18	0.76	1.88	2.30
					Year × BTB inoculation				
Y_1I_1	24.29 b	36.67 b	-	-	2.55 b	-	-	42.47 b	44.96 b
Y_1I_2	16.33 c	27.20 d	-	-	2.26 d	-	-	38.02 c	41.25 c
Y_2I_1	28.14 a	39.01 a	-	-	2.77 a	-	-	53.16 a	54.91 a
Y_2I_2	23.71 b	29.62 c	-	-	2.44 c	-	-	43.62 b	45.70 b
LSD 0.05	2.18	1.09			0.19			2.91	2.54
					Location × BTB inoculation				
S_1I_1	27.67 a	39.64 a	-	-	-	6.72 a	39.14 c	45.28 b	53.04 a
S_1I_2	24.76 b	29.07 c	-	-	-	5.94 b	39.21 c	40.07 c	44.59 bc
S_2I_1	21.21 c	36.04 b	-	-	-	4.88 c	55.73 a	50.34 a	46.83 b
S_2I_2	18.83 d	27.75 d	-	-	-	4.41 d	54.03 b	41.56 c	42.37 c
LSD 0.05	2.20	1.43				0.21	0.78	2.21	2.33

Here: Y_1 = 2019–2020; Y_2 = 2020–2021; S_1 = rainfed; S_2 = irrigated; I_1 = BTB inoculation; and I_2 = no BTB inoculation. LSD = least significant difference. Any two means sharing one letter in common within a column are statistically similar at 95% probability level. - = the relevant parameter was not significantly altered by the respective explanatory variable.

3.2. Allometric Traits

Chlorophyll index, LAI and CGR increased from 35 to 95 days after sowing (DAS) under all B application methods and BTB inoculation in irrigated and rainfed conditions (Figures 1–3). However, chlorophyll index suddenly declined after 75 DAS during 2020–2021 at both locations (Figure 1). Regarding interaction, osmopriming combined with BTB inoculation resulted in the highest chlorophyll index, LAI and CGR compared to the rest of the treatments under rainfed condition, whereas lowest values were noted for the control treatment under irrigated conditions during both years (Figures 1–3).

3.3. Yield and Related Traits

The individual and interactive effects of year, different B application methods, BTB inoculation and locations had significant effects on all yield-related traits except four-way interactions (Table 2). Number of pods per plant was significantly affected by all individual, two-way, and three-way (except for year × location × BTB inoculation and year × B application methods × BTB inoculation) interactions of year, different B application methods, BTB inoculation and locations (Table 2). Number of grains per pod were only altered by individual effects of year, B application methods, BTB inoculation and locations, whereas their all two-, three- and four-way interactions remained non-significant in this regard. Similarly, individual effects of all studied factors, two-way interaction among year and location, location, and B application methods and BTB inoculation and B application methods, and three-way interaction among year, location and B application methods and location, BTB inoculation and B application methods had a significant impact on number of grains per plant, whereas the remaining possible interactions were non-significant (Table 2).

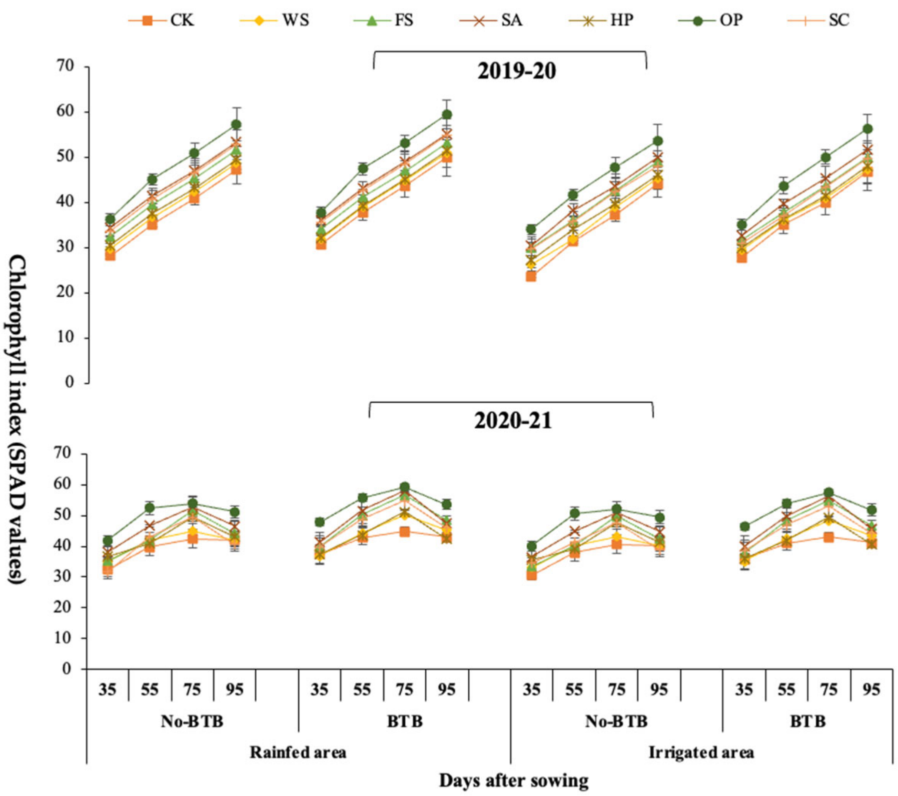

Figure 1. The impact of different boron application methods and boron-tolerant bacteria (*Bacillus* sp. MN54) on chlorophyll index (SPAD value) of desi chickpea grown under rainfed and irrigated conditions. Here: CK = control; WS = water spray; FS = foliar spray of B; SA = soil application of B; HP = hydropriming; OP = osmopriming of B; SC = seed coating of B; and BTB = boron-tolerant bacteria.

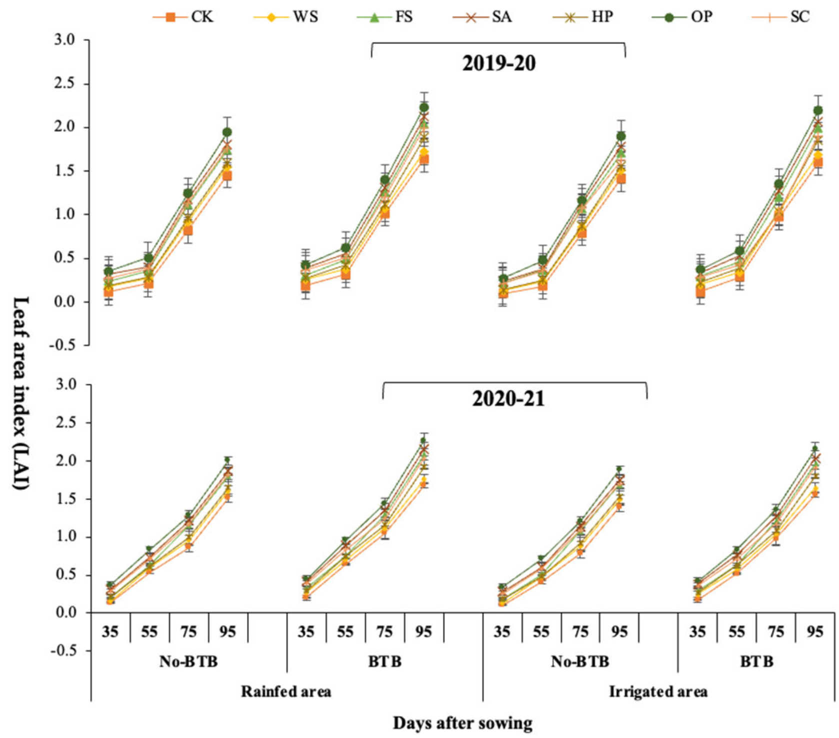

Figure 2. The impact of different boron application methods and boron-tolerant bacteria (*Bacillus* sp. MN54) on leaf area index of desi chickpea grown under rainfed and irrigated conditions. Here: CK = control; WS = water spray; FS = foliar spray of B; SA = soil application of B; HP = hydropriming; P = osmopriming of B; SC = seed coating of B; and BTB = boron-tolerant bacteria.

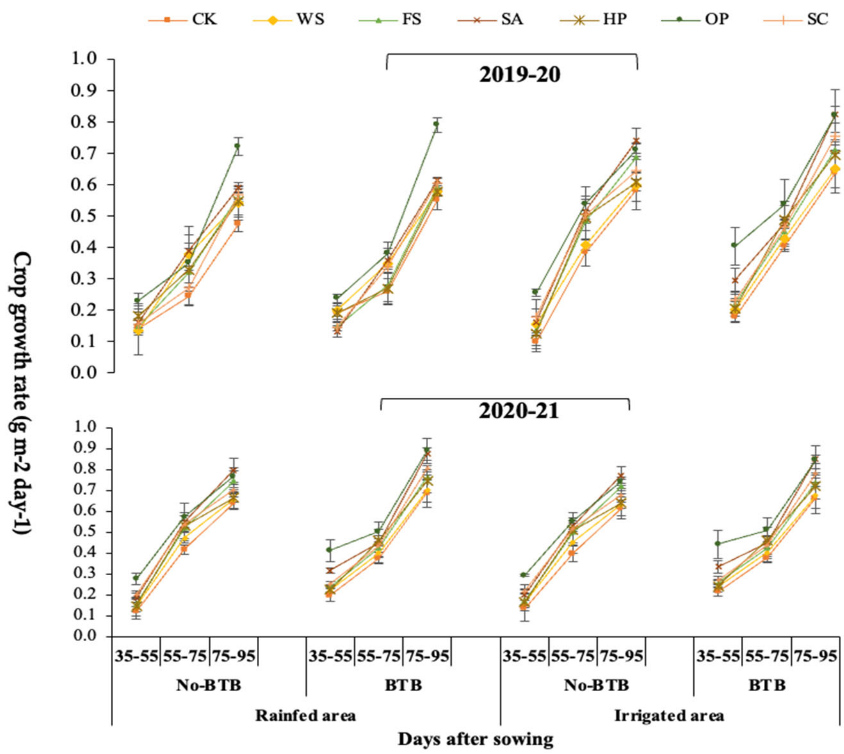

Figure 3. The impact of different boron application methods and boron-tolerant bacteria (*Bacillus* sp. MN54) on crop growth rate of desi chickpea grown under rainfed and irrigated conditions. Here: CK = control; WS = water spray; FS = foliar spray of B; SA = soil application of B; HP = hydropriming; OP = osmopriming of B; SC = seed coating of B; and BTB = boron-tolerant bacteria.

The individual effects of all factors significantly altered (except for the non-significant effect of year on harvest index) 1000-grain weight, grain and biological yields, harvest index and plant and grain B concentration. The two-way interaction among year and location, location, and B application methods and BTB inoculation and B application methods and three-way interaction among location, BTB inoculation and B application methods had a significant effect on 1000-grain weight, while the rest of the interactions remained non-significant. All possible two-way interactions (except year × B application methods and location × BTB inoculation) and three-way interaction among location, BTB inoculation and B application methods significantly affected grain yield, whereas the rest of the interactions were non-significant. Likewise, biological yield and harvest index were significantly altered by two-way interactions of year and location, B application methods with location and BTB inoculation and locations and BTB inoculation and three-way interaction of location, BTB inoculation and B application methods, whereas the remaining possible interactions proved non-significant. All two-way interactions (except B applications methods with location and BTB inoculation) and three-way interaction of location, BTB inoculation and B application methods had a significant effect on plant B concentration, while the remaining possible interactions remained non-significant. Similarly, grain B concentration was significantly altered by all two-way interactions (except B applications methods with year and BTB inoculation) and three-way interaction among year × B application methods × BTB inoculation and year × B application methods × BTB inoculation. However, the remaining interactions remained non-significant for grain B concentration (Table 2).

The higher values of yield and related traits were generally noted for 2020–2021, rainfed location, osmopriming and BTB inoculation. The year 2020–2021 recorded 9.21%, 26.97%, 3.92%, 7.88%, 8.35%, 20.25% and 16.68% higher number of pods per plant, number of grains per pod, number of grains per plant, 1000-grain weight, grain yield, biological yield, grain B concentration and plant B concentration, respectively, compared to 2019–2020. Similarly, BTB inoculation improved number of pods per plant, number of grains per pod, number of grains per plant, 1000-grain weight, grain yield, biological yield, harvest index, grain B concentration and plant B concentration by 24.92%, 12.20%, 30.86%, 5.98%, 11.65%, 10.69%, 1.71%, 14.62% and 12.92%, respectively (Table 3). Likewise, 90.82%, 46.84%, 143.99%, 18.24%, 30.32%, 37.42%, 211.68% and 192.89% improvement was recorded in number of pods per plant, number of grains per pod, number of grains per plant, 1000-grain weight, grain yield, biological yield, grain B concentration and plant B concentration, respectively, with osmopriming compared to control treatment of the study. The highest improvement in grain B concentration (242.66%) and plant B concentration (217.75%) was noted for foliar application of B compared to the control treatment of the study (Table 3).

Regarding the two-way interaction among year and location, rainfed location during 2020–2021 recorded higher values for number of pods per plant, number of grains per pod, number of grains per plant, 1000-grain weight, biological yield, and plant B concentration, whereas the irrigated location during 2020–2021 noted higher values for grain yield, harvest index and grain B concentration, whereas the irrigated location during 2019–2020 recorded the lowest values of all these traits (Table 4). Similarly, BTB inoculation during 2020–2021 recorded the highest values for number of pods per plant, grain yield, grain B concentration and plant B concentration, while no BTB inoculation during 2019–2020 recorded the lowest values of these traits. In the same way, rainfed location with BTB inoculation recorded higher values for number of pods per plant, biological yield and plant B concentration and irrigated location with BTB inoculation noted higher values for harvest index and grain B concentration, while irrigated location with no BTB inoculation recorded the lowest values of these traits (Table 4).

The highest values for number of pods and grains per plant, 1000-grain weight and grain and biological yields were noted for rainfed location with osmopriming, whereas rainfed location with foliar application of B recorded higher plant B concentration (Table 5). Similarly, osmopriming combined with BTB inoculation recorded the highest values of all yield-related traits, whereas control treatment with no BTB inoculation noted the lowest values for all yield-related traits (Table 5).

Table 5. The impact of location and BTB inoculation interactions with boron application methods on growth and yield-related traits of desi chickpea.

	Pods Plant^{-1}	Grains Plant^{-1}	1000-Grain Weight (g)	Grain Yield (t ha^{-1})	Biological Yield (t ha^{-1})	Harvest Index (%)	Plant B Concentration (mg)
			Location × B application methods				
S_1B_1	26.17 h	66.82 i	222.07 f	2.13 j	5.20 e	41.04 c	26.83 ef
S_1B_2	28.67 g	76.10 h	229.30 e	2.24 i	5.64 d	39.71 cd	26.94 de
S_1B_3	36.67 d	99.77 d	238.60 c	2.53 e	6.49 c	39.08 d	72.97 a
S_1B_4	39.83 c	112.71 c	249.60 b	2.71 c	6.99 b	38.99 d	58.99 c
S_1B_5	29.17 fg	82.02 gh	224.70 f	2.32 h	5.70 d	40.88 c	31.20 d
S_1B_6	47.00 a	136.50 a	260.80 a	2.91 a	7.80 a	37.42 e	65.84 b
S_1B_7	33.00 e	90.66 ef	227.55 e	2.41 fg	6.50 c	37.09 e	58.95 c
S_2B_1	21.53 i	39.55 k	210.49 h	2.29 h	4.25 h	53.74 b	17.91 h
S_2B_2	24.91 h	49.73 j	216.78 g	2.45 f	4.35 h	56.19 a	19.23 gh
S_2B_3	31.00 f	76.54 h	232.06 d	2.60 d	4.65 g	55.82 a	69.19 ab
S_2B_4	36.55 d	94.24 de	238.18 c	2.71 c	4.93 f	55.11 ab	59.00 c
S_2B_5	30.33 fg	65.61 i	215.53 g	2.37 g	4.37 h	54.15 b	22.57 fg
S_2B_6	44.02 b	123.05 b	250.65 b	2.86 b	5.21 e	55.07 ab	65.20 b
S_2B_7	34.53 e	85.92 fg	232.96 d	2.56 de	4.74 g	54.08 b	59.06 c
LSD 0.05	1.88	6.75	4.76	0.12	0.17	1.43	4.30
			BTB inoculation × B application methods				
I_1B_1	27.03 i	65.64 g	223.24 g	2.40 g	4.91 h	50.01 a	-
I_1B_2	30.33 g	75.30 f	229.64 f	2.48 f	5.16 g	49.27 ab	-
I_1B_3	38.38 d	102.73 d	242.62 d	2.73 c	5.92 cd	47.59 cde	-
I_1B_4	43.42 b	118.60 b	252.51 b	2.84 b	6.47 b	45.29 fgh	-
I_1B_5	36.03 e	93.61 e	228.16 f	2.52 f	5.34 ef	48.34 bcd	-
I_1B_6	50.26 a	148.07 a	263.09 a	3.03 a	7.01 a	45.17 gh	-
I_1B_7	39.44 cd	105.08 cd	235.44 e	2.63 d	5.78 d	46.35 efg	-
I_2B_1	20.67 k	40.73 i	209.33 j	2.02 j	4.55 j	44.76 h	-
I_2B_2	23.24 j	50.53 h	216.44 h	2.20 i	4.82 hi	46.63 ef	-
I_2B_3	29.29 gh	73.58 f	228.04 f	2.40 g	5.23 fg	47.30 de	-
I_2B_4	32.97 f	88.35 e	235.27 e	2.58 e	5.45 e	48.81 abc	-
I_2B_5	23.85 j	54.01 h	212.07 i	2.17 i	4.73 i	46.69 ef	-
I_2B_6	40.76 c	111.48 c	248.36 c	2.73 c	6.00 c	47.33 de	-
I_2B_7	28.09 hi	71.50 fg	225.07 g	2.34 h	5.46 e	44.82 h	-
LSD 0.05	1.93	5.98	3.67	0.16	0.15	1.40	

Here: S_1 = rainfed; S_2 = irrigated; I_1 = BTB inoculation; I_2 = no BTB inoculation; B_1 = control (0.00); B_2 = water spray; B_3 = foliar spray at 0.05% B; B_4 = soil application; B_5 = hydropriming; B_6 = osmopriming 0.001% B; and B_7 = seed coating 1.5/kg seed. LSD = least significant difference. Any two means sharing one letter in common within a column are statistically similar at 95% probability level. - = the relevant parameter was not significantly altered by the respective explanatory variable.

The year × location × boron application methods' interaction was only significant for number of pods and grains per plant and plant B concentration (Table 6). Rainfed location during 2020–2021 with osmopriming recorded the highest values for number of pods and grains per plant, while rainfed location with foliar application of B during 2020–2021 recorded the highest plant B concentration. The lowest values of these traits were recorded for the control treatment in the irrigated location during 2019–2020 (Table 6).

Table 6. The impact of year × location × boron application methods' interaction on number of pods and grains per plant and plant boron concentration of desi chickpea.

	2019–2020		**2020–2021**	
B Application Methods	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**
Number of pods per plant				
Control (0.00)	24.67 kl	21.07 n	27.67 ij	22.00 mn
Water spray	27.17 jk	24.32 lm	30.17 hi	25.50 jkl
Foliar spray at 0.05% B	35.17 fg	30.66 h	38.17 de	31.33 h
Soil application	38.33 de	35.93 efg	41.33 c	37.17 ef
Hydropriming	27.67 ij	30.42 h	30.67 h	31.00 h
Osmopriming 0.001% B	45.50 b	39.98 cd	48.50 a	48.05 ab
Seed coating 1.5/kg seed	31.50 h	34.73 fg	34.50 g	34.33 g
LSD 0.05	9.55			
Number of grains per plant				
Control (0.00)	45.38 no	34.70 p	88.27 ghi	44.40 o
Water spray	57.18 lm	45.11 no	95.02 efg	54.35 mn
Foliar spray at 0.05% B	88.28 ghi	71.43 jk	111.25 c	81.65 hi
Soil application	101.13 de	87.67 ghi	124.28 b	100.82 de
Hydropriming	65.05 kl	64.27 kl	98.98 def	66.95 k
Osmopriming 0.001% B	127.05 b	107.88 cd	145.95 a	138.22 a
Seed coating 1.5/kg seed	80.28 ij	81.26 hi	101.04 de	90.58 fgh
LSD 0.05	10.12			
Plant B concentration				
Control (0.00)	15.67 i	17.66 hi	38.00 g	18.17 hi
Water spray	19.45 hi	19.26 hi	34.43g	19.19 hi
Foliar spray at 0.05% B	71.91 ab	65.92 bc	74.02 a	72.47 a
Soil application	54.96 f	56.53 ef	63.03 cd	61.48 cde
Hydropriming	23.61 h	22.73 h	38.79 g	22.41 h
Osmopriming 0.001% B	60.09 cdef	60.37 cdef	71.59 ab	70.04 ab
Seed coating 1.5/kg seed	56.79 ef	58.57 def	61.10 cde	59.55 def
LSD 0.05	6.09			

LSD = least significant difference. Any two means sharing one letter in common within a column are statistically similar at the 95% probability level.

Regarding three-way interactions among location, BTB inoculation and B application methods, osmopriming at rainfed location with BTB inoculation recorded the highest values of number of pods and grains per plant. Similarly, foliar application of B at rainfed location with BTB inoculation noted higher plant B concentration (Table 7). The lowest values of these traits were noted for the control treatment with no BTB inoculation at the irrigated location (Table 7).

Table 7. The impact of location × BTB inoculation × boron application methods' interaction on number of pods and grains per plant and plant boron concentration of desi chickpea.

	Rainfed		Irrigated	
	BTB	**No BTB**	**BTB**	**No BTB**
Number of pods per plant				
Control (0.00)	31.17 h	21.17 mn	22.90 lm	20.17 n
Water spray	34.83 g	22.50 lmn	25.83 jk	23.98 kl
Foliar spray at 0.05% B	42.17 bc	31.17 h	34.58 g	27.41 ij
Soil application	43.83 b	35.83 fg	43.00 b	30.10 h
Hydropriming	35.50 g	22.83 lm	36.55 efg	24.87 jkl
Osmopriming 0.001% B	50.83 a	43.17 b	49.68 a	38.35 def
Seed coating 1.5/kg seed	39.17 de	26.83 ij	39.72 cd	29.35 hi
LSD 0.05	9.63			
Number of grains per plant				
Control (0.00)	84.73 fg	56.81 l	48.92 kl	32.55 m
Water spray	93.78 ef	89.87 jk	58.42 jk	42.65 l
Foliar spray at 0.05% B	115.58 bc	114.77 fg	83.95 g	63.20 ij
Soil application	122.43 b	84.50 bc	102.98 de	73.72 h
Hydropriming	102.73 de	145.41 fg	61.30 ij	46.72 l
Osmopriming 0.001% B	150.73 a	102.96 de	122.27 b	100.69 de
Seed coating 1.5/kg seed	107.20 cd	56.81 de	74.12 h	68.88 hi
LSD 0.05	9.55			
Plant B concentration				
Control (0.00)	32.91 g	20.76 hij	20.53 hij	15.29 j
Water spray	32.57 g	21.31 hij	22.15 hi	16.30 ij
Foliar spray at 0.05% B	75.05 a	70.88 ab	71.12 ab	67.26 bcd
Soil application	63.78 cd	54.20 f	63.15 cd	54.85 f
Hydropriming	36.07 g	26.33 h	22.94 h	22.20 hi
Osmopriming 0.001% B	68.37 bc	63.31 cd	66.29 bcd	64.11 cd
Seed coating 1.5/kg seed	62.56 cde	55.33 f	61.58 de	56.54 ef
LSD 0.05	6.21			

LSD = least significant difference. Any two means sharing one letter in common within a column are statistically similar at the 95% probability level.

Similarly, osmopriming with BTB inoculation at the rainfed location recorded the highest values of 1000-grain weight, and grain and biological yields, whereas control treatment with no BTB inoculation recorded the lowest values of these traits (Table 8).

Table 8. The impact of location × BTB inoculation × boron application methods' interaction on yield and related traits of desi chickpea.

Treatments	Rainfed		Irrigated		Rainfed		Irrigated	
	BTB	No BTB	BTB	No BTB	BTB	No BTB	BTB	No BTB
	1000-Grain Weight (g)				Grain Yield (t ha^{-1})			
Control (0.00)	228.67 i	215.47 m	217.81 lm	203.18 o	2.25 k	2.01 n	2.55 h	2.03 n
Water spray	234.90 fg	223.70 k	224.38 jk	209.18 n	2.34 j	2.13 m	2.62 fg	2.28 k
Foliar spray at 0.05% B	245.73 d	231.47 ghi	239.51 e	224.61 jk	2.72 de	2.35 j	2.73 d	2.46 i
Soil application	258.43 bc	240.77 e	246.58 d	229.78 hi	2.84 c	2.58 gh	2.84 c	2.59 gh
Hydropriming	228.13 ij	221.27 kl	228.18 ij	202.88 o	2.48 i	2.17 lm	2.56 gh	2.18 lm
Osmopriming 0.001% B	266.77 a	254.83 c	259.41 b	241.88 e	3.07 a	2.75 d	3.00 b	2.72 de
Seed coating 1.5/kg seed	238.10 ef	217.00 m	232.78 gh	233.15 gh	2.59 gh	2.23 kl	2.67 ef	2.45 i
LSD 0.05	5.12				0.06			
	Biological yield (t ha^{-1})				Harvest index (%)			
Control (0.00)	5.48 gh	4.92 i	4.33 klm	4.17 m	41.09 hi	40.99 hij	58.93 a	48.54 g
Water spray	5.87 f	5.41 gh	4.46 jkl	4.24 lm	39.92 hijk	39.49 hijk	58.61 a	53.76 def
Foliar spray at 0.05% B	6.96 c	6.03 ef	4.88 i	4.43 jkl	39.17 ijkl	38.99 jkl	56.02 bc	55.61 bcd
Soil application	7.61 b	6.37 d	5.33 h	4.54 jk	37.34 lm	40.63 hij	53.24 ef	56.99 ab
Hydropriming	6.13 e	5.27 h	4.55 j	4.18 m	40.48 hijk	41.29 h	56.21 b	52.09 f
Osmopriming 0.001% B	8.46 a	7.13 c	5.55 g	4.86 i	36.28 mn	38.56 kl	54.06 cdef	56.09 b
Seed coating 1.5/kg seed	6.54 d	6.47 d	5.03 i	4.45 jll	39.68 hijk	34.51 n	53.03 f	55.13 bcde
LSD 0.05	0.22				2.01			

LSD = least significant difference. Any two means sharing one letter in common within a column are statistically similar at the 95% probability level.

3.4. Economic Analysis

The economic analysis described that B application methods combined with BTB inoculation positively improved the net benefit and benefit–cost ratio during both years of study under rainfed and irrigated conditions (Table 9). The highest net benefit (USD 2068.5 ha^{-1}) and benefit–cost ratio (3.7%) were obtained under osmopriming combined with BTB inoculation followed by soil application (Table 9). The BTB inoculation significantly improved net benefits and benefit–cost ratio compared with no-BTB inoculation (Table 9).

Table 9. Effects of B application methods along with BTB strain on economic analysis of desi chickpea during 2019–2020 and 2020–2021.

Treatments		Rainfed Condition								Irrigated Condition							
		2019–2020				2020–2021				2019–2020				2020–2021			
		Total Cost (USD ha^{-1})	Gross Income (USD ha^{-1})	Net Income (USD ha^{-1})	BCR %	Total Cost (USD ha^{-1})	Gross Income (USD ha^{-1})	Net Benefit (USD ha^{-1})	BCR%	Total Cost (USD ha^{-1})	Gross Income (USD ha^{-1})	Net Benefit (USD ha^{-1})	BCR%	Total Cost (USD ha^{-1})	Gross Income (USD ha^{-1})	Net Benefit (USD ha^{-1})	BCR%
No-BTB	Control (0.00)	695.40	1696.1	1000.7	2.4	722.66	1801.1	1078.4	2.5	710.8	1661.3	950.5	2.3	746.5	1861.4	1114.9	2.5
	Water spray	703.11	1799.1	1095.9	2.6	739.60	1903.4	1163.8	2.6	727.8	1878.8	1151.0	2.6	763.5	2078.8	1315.4	2.7
	Foliar spray	738.54	1987.5	1249.0	2.7	823.25	2091.9	1268.6	2.5	811.4	2041.2	1229.8	2.5	847.1	2241.2	1394.1	2.6
	Soil application	744.71	2194.8	1450.1	2.9	872.55	2299.2	1426.6	2.6	860.7	2148.4	1287.7	2.5	896.4	2348.5	1452.1	2.6
	Hydropriming	704.65	1835.3	1130.6	2.6	731.90	1939.7	1207.8	2.7	720.1	1791.8	1071.8	2.5	755.8	1991.9	1236.1	2.6
	Osmopriming	728.20	2336.3	1608.1	3.2	772.40	2440.7	1668.3	3.2	760.6	2267.3	1506.7	3.0	796.3	2467.4	1671.1	3.1
	Seed coating	703.11	1885.7	1182.6	2.7	780.11	1990.1	1210.0	2.6	768.3	2032.5	1264.2	2.6	804.0	2232.5	1428.5	2.8
BTB	Control (0.00)	701.64	1902.0	1200.3	2.7	728.89	2006.4	1277.5	2.8	717.0	2102.0	1385.0	2.9	752.8	2336.9	1584.1	3.1
	Water spray	709.34	1981.7	1272.4	2.8	745.84	2086.1	1340.3	2.8	734.0	2157.1	1423.1	2.9	769.7	2392.0	1622.3	3.1
	Foliar spray	744.78	2313.7	1568.9	3.1	829.48	2418.1	1588.6	2.9	817.6	2258.6	1441.0	2.8	853.4	2493.5	1640.1	2.9
	Soil application	750.94	2415.2	1664.2	3.2	878.78	2519.5	1640.8	2.9	866.9	2348.5	1481.5	2.7	902.7	2583.3	1680.7	2.9
	Hydropriming	710.88	2100.6	1389.7	3.0	738.14	2205.0	1466.8	3.0	726.3	2107.8	1381.5	2.9	762.0	2342.7	1580.7	3.1
	Osmopriming	734.44	2493.5	1759.0	3.4	778.64	2847.2	2068.5	3.7	766.8	2490.6	1723.8	3.2	802.5	2725.4	1922.9	3.4
	Seed coating	709.34	2199.2	1489.8	3.1	786.34	2303.5	1517.2	2.9	774.5	2200.6	1426.1	2.8	810.2	2435.5	1625.2	3.0

Here: BCR = benefit–cost ratio; ha = hectare.

4. Discussion

This two-year study revealed that B application through different methods and BTB inoculation under irrigated and rainfed conditions significantly improved root nodulation, productivity, quality and biofortification of desi chickpea (Figures 1–3; Tables 3–9). These findings are supported by Khanam et al. [43] who reported that chickpea grain yield was significantly increased by B application. We further noticed that B application through osmopriming and seed coating (seed treatment), soil application and foliar application performed better, when combined with BTB inoculation and significantly improved morphological parameters, grain yield and grain B concentration. In another study, B application through seed priming and seed coating improved early seedling growth of rice [44]. Osmopriming proved better in improving growth and yield-related traits. Osmopriming enables delayed, osmotic fluid rehydration of seeds with the goal of triggering early germination-related metabolic processes without radicle protrusion. The quicker germination and better growth in osmopriming probably enabled plants to utilize resources more efficiently, which resulted in higher productivity. The 2nd year of the study noted higher values of all traits. Since the experiment was repeated on the same experimental field, B availability and higher BTB density during second year resulted in higher values of yield and related traits. Similarly, rainfed location resulted in better productivity due to wider adaptability of chickpea to rainfed conditions in the country. The better yield and related traits in BTB inoculation are owed to the bacterial activity which improved B supply compared to no BTB inoculation.

Increased grain yield under different B application methods might be linked to higher root nodulation (Table 3). Improvement in root nodules was higher with BTB inoculation in rainfed conditions compared with untreated seeds (Table 3). Soil microbes improve nutrient supply by releasing several organic acids, dissolving fixed minerals and making them available to plants [32]. The higher nodulation in BTB inoculation might be the reason for improved chickpea performance. The number of nodules per plant increased with the increasing rates of B application at normal amount (1.5 kg B ha^{-1}) [45]. The same pattern was noted in our study that different B application methods and BTB inoculation significantly improved matter production and grain productivity (Figure 3; Tables 3–8). Farooq et al. [46] revealed that osmopriming enhanced fresh weight and root elongation in rice. Soil applied B at 0.25 mg kg^{-1} soil coupled with BTB strain resulted in the highest dry weight and plant height of chickpea [20].

Chickpeas are mostly grown in desert areas, where soils are mostly deficient in micronutrients, particularly B. Boron is required by plants in minute quantity; however, metabolic activities are significantly altered by B-deficiency. Boron stabilizes plant cell wall, membrane integrity and sugar transport, and improves utilization of calcium and nitrogen [47,48]. In our study, chlorophyll index, leaf area index and dry matter accumulation are increased by B application combined with BTB inoculation. Chlorophyll index and leaf area index directly linked with photosynthesis. Higher chlorophyll index and leaf area index resulted in more green parts which enhanced the production potential of the crop. Yamori et al. [49] reported that leaf photosynthesis was the most significant factor for attaining higher grain yield and biomass. Ali et al. [50] reported that there was an association between LAI and total grain yield, and that higher LAI produced higher yield. Lower chlorophyll index, poor growth rate and dry matter accumulation was observed for no B application (Figures 1–3). Boron deficiency during reproductive stage lowers plant height, reduces dry matter production and interrupts photosynthesis [51]. The same results were obtained in our study, where growth, allometric and yield related parameters were reduced in the treatments receiving no B application (Figures 1–3, Tables 3–8).

Improvement in grain yield was higher under B application with osmopriming as compared to no B application (Table 6). This might be due to improved yield-related parameters, i.e., number of pods per plant, grains per pod and 1000-grain weight. Osmopriming positively improved plant height compared with untreated seeds (Table 3). Bangar et al. [52] also reported increase in plant height in soybean (*Glycine max* L.) with B application. Number of pods per plant, grains per pod and 1000-grain weight increased under 0.001% B priming compared with control [32]. Chickpea grain yield under rainfed condition was higher than irrigated areas. Pod formation and grain quality was improved under B application because it takes part in assimilated partitioning. Assimilates are stored in the stem and leaves (vegetative portion) of plants in a large amount and then translocate into reproductive parts [53].

The higher number of grains per pod, pods per plant and 1000-grain weight was observed under rainfed condition with B application through osmopriming, which ultimately increased grain yield (Tables 5 and 6). Boron is an essential micronutrient which plays a key role in sugar transport from source to sink, helps in carbohydrate metabolism, improves pollen fertility and flower life; thus, improves yield-related traits. Similarly, higher chickpea yield was recorded in this study with B application. According to Hussain et al. [26], seed coating with 1.5 g B kg^{-1} seed positively enhanced number of seed per plant which further improved grain production. Boron application methods (seed priming, coating, soil, and foliar application) proved helpful in improving chickpea production and grain quality. The highest B grain concentration was recorded under foliar application compared with other levels (Table 3). The BTB inoculation combined with B application increased chickpea production compared to no BTB inoculation. The BTB inoculation exerted significantly improved 1000-grain weight, grain yield and biological yield compared to no BTB inoculation. These results confirmed the findings of Mehboob et al. [20] who stated that number of branches $plant^{-1}$, number of grains pod^{-1} and 1000-grain weight of chickpea showed improvement with the application of BTB strain MN54. According to Ullah et al. [54], Zn application through soil application or seed coating coupled with plant growth promoting bacteria positively improved productivity, profitability and grain quality of desi chickpea. Boron nutrition as osmopriming in chickpea resulted in higher grain yield as compared to control with an average yield difference of 30%. Another study conducted by Dar [55] reported that chickpea yield was increased by 54% with the application of B at the rate of 1 kg ha^{-1}. Samreen et al. [37] reported that BTB strain MN-54 improved nutrient supply in canola (*Brassica napus*) leading to its improved growth. Therefore, higher productivity of chickpea due to B application combined with BTB application in this study was linked with continuous and higher B supply.

Boron deficiency and toxicity are injurious for plant growth as well as human health since it is required in a lower amount. It was confirmed by this study that chickpea growth and productivity are affected by B-deficiency. These results agree with previous studies reporting both increased and reduced yield responses by various chickpea genotypes to B-deficiency [56].

5. Conclusions

Boron application by different methods along with inoculation of boron-tolerant bacteria *Bacillus* sp. strain MN-54 considerably enhanced nodule population, dry matter accumulation, grain yield, quality and biofortification of desi chickpea under rainfed conditions. Similarly, osmopriming with 0.001% B solution combined with boron-tolerant bacteria inoculation improved chickpea grain production. However, higher grain B concentration was observed under foliar application of 0.025% B combined with boron-tolerant bacteria inoculation. Hence, B application through osmopriming combined with BTB inoculation seemed a pragmatic option to boost production, higher economic returns, and grain B concentration of desi chickpea under irrigated and rainfed conditions.

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