Can the new boron-fertilization method improve the system productivity of rice (Oryza sativa L.) – mustard (Brassica juncea L.) cropping system under upland calcareous soils?

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Research

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Abstract

Background

Calcareous soils are highly deficient in boron (B) which has become one of the most important deficient micronutrients in Indian soil after zinc (Zn). For various rice-based cropping systems, B-fertilization is essential for increasing crop productivity and the biofortification of the crop, thus suitable soil application protocol for B-fertilization are required for B-deficient soils.

Results

In a six years-long experiment, different rates of B application viz. 0.5, 1.0, 1.5, and 2.0 kg ha$^{-1}$ y$^{-1}$ were evaluated to determine the effects of three different modes of B fertilization viz. applied only in the first year, in alternating years, and every year in rice (Oryza sativa L.) – Indian mustard (Brassica juncea L.) cropping system. It was observed that the application of B at 1.5 kg ha$^{-1}$ in every year or 2 kg ha$^{-1}$ in alternate years resulted in the highest yield of rice and mustard as well as the system productivity of the rice–mustard cropping system. Application of 2 kg ha$^{-1}$ B in the initial year showed the maximum B-uptake by rice, while, application of 1.5–2.0 kg ha$^{-1}$ B in every year resulted in the maximum B-uptake by the mustard crop.

Conclusion

Application of B at 2 kg ha$^{-1}$ in alternate years or 1.5 kg ha$^{-1}$ in every year was the best B-application protocol under rice–mustard cropping system in B-deficient calcareous soils for ensuring the best system productivity of rice–mustard cropping system and B-availability in soil.

Background

Boron (B) is a limiting factor in crop productivity in rice-based systems [1]. Such deficiency of boron has emerged as an important micronutrient problem in Indian soils and crops next to zinc. Analysis reports of soil samples indicated that deficiency of boron has been found up to 84% with a mean of 33% and upland calcareous areas in India are prone to B deficiency [2]. Various soil factors including pH, organic matter, clay minerals, sesquioxides (Fe and Al oxides), carbonates, and tillage significantly influence the plant availability to B, the content of soil extractable B and different B fractions transformations in soil [3],[4],[5]. Retention of B in soil constituents is favoured by an increase in pH [6],[7]. Calcareous soils with light (sandy) in texture and having low organic matter content are suffered from boron deficiency [8]. An increase in calcium carbonate content raises soil pH limiting the availability of B by serving as a sink for B in soil [9],[10] where it is involved in surface adsorption on a huge portion of the soluble B and thus decreases its availability for plant uptake [11].
Boron helps in the synthesis of oil and protein. Restricted fruit development and flowering have been observed by the deficit of B. However, the requirement of B varies with the species, crops, and phonological stages of crop growth [12]. There are multitudinous reports on the positive response of mustard to B fertilization [13],[14],[15]. Such a phenomenon strongly indicates a greater degree of sensitivity to B application in the mustard crop. B deficiency in soil decreases rice productivity through increment in grain sterility, decrement in the number of productive tillers, lesser chloroplasts, and disruption in grain cooking quality with decreased net assimilate rates [1],[16]. They reported that the B-fertilization in different rice-based systems favoured for improvement in productivity by reduction of yield losses, and improvement in cooking quality of grain. Thus, the judicial use of B fertilizer is very important. On the other hand, the rice–mustard cropping system is of great importance in eastern India for diversification in cereal-based rice-wheat cropping system [17] and making the country self-sufficiency in oil production [18].

Boron is found in different components of the soil, including soil solution, organic matter (OM), and minerals. B-fractionation of soils insights for qualitative and quantitative importance of fractions of B [19]. Fractionation helps in defining and measuring different types of total Soil B which have the potential for predicting bioavailability, laxity, dynamics, soil processing, and environmental impacts between various chemical types [20],[21]. Soil Boron is known to be distributed through geochemical formes, e.g. fine-soluble, oxide bound, specifically adsorbed, organically bound and residue forms that have major variations in bioavailability, mobility and chemical behaviour of soils and that can be transformed under certain conditions. In order to understand its chemistry in soils, fractionation of B into these types along with knowledge about the chemistry of B is very important [22]. Boron in soil solution is readily accessible for plant absorption, but the retention of B in soil solution is a crucial feature of plant nutrition and is regulated in other soil fractions by the pools of B and their equilibrium with soil solution [23]. The positive effects of applied B on successive crops in a growing system can last over varying periods, as B has been transformed into different forms in calcareous soil. It is often difficult to estimate the residual effect of the B fertiliser applied at various concentrations for different times, to make recommendations for B applications for a cropping system. It is therefore important to be aware of the rate and frequency of B application for the important rice–mustard cropping system in calcareous soils.

Concerning all of these, a six-year experiment was designed with the objectives (i) to establish a suitable B-fertilization protocol for higher yield of rice and mustard, (ii) to study the B transformation into various extractable fractions, and (iii) to assess the interrelation between readily soluble B with yield of the crops and B-uptake.

**Materials And Methods**

**Experimental site**

A six-year field investigation with rice–mustard cropping system was conducted at the experimental farm of Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India (25°94’ N, 85°67’ E, and 52 m
above msl). The field observation was initiated in 2012. The soil of the experimental field is sandy loam soil (typic calciorhent in the soil taxonomy by USDA).

**Experimental soils**

The initial characteristics of the experimental site in surface soil (0–15 cm) having pH of 8.41 (1:2 soil water suspension); electrical conductivity of 0.62 dSm$^{-1}$ (1:2 soil water suspension); free CaCO$_3$ of 34%; organic carbon of 4.23 g kg$^{-1}$; available N of 188 kg ha$^{-1}$; available P$_2$O$_5$ of 11.3 kg ha$^{-1}$; available K$_2$O of 88.3 kg ha$^{-1}$; and available S of 11.6 kg ha$^{-1}$. The available micronutrients, B, Zn, Cu, Fe, Mn at the beginning of the experiment were 0.41 mg kg$^{-1}$, 0.66 mg kg$^{-1}$, 2.46 mg kg$^{-1}$, 16.28 mg kg$^{-1}$, and 4.68 mg kg$^{-1}$, respectively.

**Climatic condition**

The climate of the site comprises mainly three seasons, i.e., rainy (June to September), winter (October to February), and summer (March to May) and mean maximum and minimum temperatures on monthly basis varies between 23.8–36.8 and 9.1–27.2°C, respectively. Frequent droughts and floods are common and characterized by hot and humid summers and cold winters with an average rainfall of 1200 mm y$^{-1}$, most of which occurs during July-September. The soil was potentially B deficient in upland calcareous. The area of each plot was 12 m$^2$ (4 m x 3 m).

**Experimental treatments, design and procedures**

The experiment consisted of three different combinations of B application in four rates (0.5, 1.0, 1.5, and 2.0 kg ha$^{-1}$) in the initial year, alternate year, and every year with a control plot of B where no B was applied. B was applied to the soil as Borax {Na$_2$[B$_4$O$_5$(OH)$_4$]$_8$H$_2$O} to the rice–mustard cropping system. All treatments were arranged in a randomized complete block design (RCBD) and repeated three times.

During the six years of study, B was applied only once in the initial year applied plot; thrice in the alternate years applied plots; and six times in every year mode of applications. The present study was undertaken during the sixth year (2017–2018) in both rice and mustard crops. Rice crop of locally popular variety, *Rajendra Bhagwati* was transplanted during late June and was harvested in late October while the mustard (variety, *Rajendra Sufalam*) was sown in early November and was harvested in late February in each year of the study. Recommended package of practices was followed to grow both of these crops.

The fertilizers urea at 211 kg ha$^{-1}$, diammonium phosphate (DAP) at 130 kg ha$^{-1}$, and muriate of potash (MOP) at 100 kg ha$^{-1}$ were applied as sources of N, P, and K, respectively to rice in each experimental plot. For mustard, the rates of urea, DAP and MOP were 96 kg ha$^{-1}$, 87 kg ha$^{-1}$, and 100 kg ha$^{-1}$ in each plot.

**Extraction procedures**

**Available B and extraction of B fractions in soil**
Finely ground and 2 mm sieved air-dried soil samples were used for B fractions extraction. The sequential extraction procedure as proposed by [23],[24] and modified by Datta et al. [25] was followed to determine the different sequential B fractions in the soil after the harvest of mustard i.e. at the end of the six years of experimentation.

**Readily soluble fraction (Solution plus Non-specifically adsorbed)**

The supernatant solution was filtered using Whatman 42 No. filter paper, which was aggregated with 5 gm of soil adding 10 ml of 0.01 M CaCl$_2$ (1:2 soil: solvent) in 50 ml centrifuges of polyethylene shaken for 16 h and centrifuged for 30 min at 10,000 rpm. Clear extracts with azomethine-H were determined in B [26].

**Specifically adsorbed**

The residue from the above step was then extracted with 10 ml of 0.05 M KH$_2$PO$_4$ by shaking for 1 h. After centrifugation, B was measured in the clear extract as described in the previous step.

**Oxide bound**

With 0.2 M NH$_4$-oxalate (1:4 ground: solution) of 20 ml, the residue from the previous step has been removed by shaking for 4 h at the pH of 3.25. A 14 ml aliquot from the extract was taken into a 50 ml beaker to remove a slightly yellowish to a reddish colour because of Fe and the minor dissolution of organic matter colour. The content of the dissolved Fe as Fe(OH)$_3$ was held on a hot plate and 2 ml of 5 N NaOH solution had been applied. After the aliquot in the beaker had been weighed, weights were decreased by adding distilled water. The suspension has been filtered by the filtering substance Whatman No. 42 and Fe has been removed. A 9 ml aliquot was taken from the filtrate and heated on a hotplate at 135 ± 5 °C to kill the organic material by adding 50 ml Teflon beaker. 4 ml concentrated H$_2$SO$_4$ and 1 ml HClO$_4$ (60%) was applied. Once the volume has been decreased to about 6 ml, the HClO$_4$ has been additionally applied to the solution by an increase of 0.5 ml. The material was then converted into a polythene tube of 15 ml and the final volume was 6 ml. B in the clear extract was calculated with a carmine method after centrifugation at 10,000 rpm for 15 min [26].

**Organically bound**

The above residue was removed by shaking for 24 h and then filtering through Whatman No. 42 using 20 ml of 0.5 M NaOH. The method described by Datta et al. [25] was used to eliminate the colour from the filtrate. Carmine [26] determined the B in the simple extract.

**Residual fraction**

The residue was dried and ground from the previous phase. 1 g sub-sample has been taken into a 50 ml of Teflon beaker with a small volume of H$_2$SO$_4$, HF of 5 ml (40%), and HClO$_4$ of 0.5 ml (60%) [27]. At 135 ± 5 °C, the beaker was put on a thermal plate and the ability was reduced to approximately 3 ml. The
heating was then applied and proceeded by adding 5 ml concentrated $\text{H}_2\text{SO}_4$ and 5 ml of HF (40%). In increments of 2–5 ml, additional HF was applied before the soil was completely digested. Depending on color intensity in the extract, a clear extract was added 3 to 5 ml of $\text{HClO}_4$ (60%) after digestion. The heating and removal of HF and $\text{HClO}_4$ reduced the volume to 3–4 ml. The volume was up to 25 ml and the material moved into centrifuge tubes. The obvious supernatant was calculated with carmine after centrifugation of 10,000 rpm.

**Analysis of plant samples for the estimation of B content and uptake**

During harvest, grain or seed and straw or stover yields for each experimental plot were reported and composite grain/seed and straw/stover samples from each plot were collected. The samples were washed with deionized (DI) water with 0.1 M HCl. Additional moisture was removed. They were placed in new bags of paper and dried at 70 °C in the oven. Wiley Millground weight samples have been diluted by adding distilled water and the material is filtered through Whatman no. 42 filter papers and the final volume is provided at 50 ml by the combination of concentrated $\text{HNO}_3$ and $\text{HClO}_4$ (ratio 9:4).

A 20 ml B free tube and a vortexed tube were added with 5 ml sample aliquot, 2 ml ammonium acetate buffer (pH 5.5), and 2 ml 0.02 M EDTA. The tube was once more vortexed and assisted for 1 h at 25°C, and vortexed again after 1 ml of azomethine-H reagent had been applied (0.9% azomethine-H plus 2% ascorbic acid solution) and the reading was taken at 420 nm with a spectrophotometer (Systronics 2203) [28]. The current concentration was determined using the standard curve which consists of the observed x-axis boron concentration and y-axis absorption.

The uptake of boron by rice and mustard crop was estimated as:

\[
\text{Boron uptake (g ha}^{-1}) = \frac{\text{Boron concentration (mg kg}^{-1}) \times \text{Dry matter (g)}}{10}
\]

The Rice equivalent yield (t ha\(^{-1}\)) of the rice–mustard system was the average rice yield and rice equivalent yield of mustard. Rice equivalent yield of mustard was determined as follows:

\[
\text{Rice equivalent yield (Mg ha}^{-1}) = \frac{\text{Mustard yield (Mg ha}^{-1}) \times \text{MSP of mustard (INR kg}^{-1})}{\text{MSP of rice (INR kg}^{-1})}
\]

where, MSP of rice and mustard denotes the minimum support price.

**Statistical analysis**

The data are analyzed statistically using the randomized complete block design process [29]. All the data are subjected to the analysis of variance (ANOVA). The interpretation of multiple variance sources by error mean square of the Fisher Snedecor’s ‘F’ test at probability level 0.05. For data processing, Windows SPSS 16.0 (SPSS Inc. Chicago, IL) and Microsoft Excel (Microsoft Corp., Pullman, WA) software were used. All the graphs were drawn using Microsoft Excel software (Microsoft Corp., Pullman, WA).
## Results

### Yields of rice and mustard

Compared with control, all the B fertilization practices significantly increased the rice grain and straw yields. However, there was no such significant impact of different rates and methods of B application on grain yield of rice, while the straw yield of rice was significantly varied with the different B-application protocols (Table 1).

| Treatments                      | Rice yield (Mg ha⁻¹) | Mustard yield (Mg ha⁻¹) |
|---------------------------------|----------------------|-------------------------|
|                                 | Grain | Straw | Seed | Stover |
| 0.5 kg B ha⁻¹ during first year | 3.94a | 4.73d | 0.95fg | 2.97cd |
| 1.0 kg B ha⁻¹ during first year | 3.92a | 4.96cd | 0.87g | 3.12abcd |
| 1.5 kg B ha⁻¹ during first year | 4.18a | 5.03bcd | 1.00fg | 3.30abcd |
| 2.0 kg B ha⁻¹ during first year | 4.29a | 5.74ab | 0.96fg | 3.18abcd |
| 0.5 kg B ha⁻¹ at alternate years | 4.47a | 5.84a | 1.18ef | 2.99bcd |
| 1.0 kg B ha⁻¹ at alternate years | 4.28a | 5.02bcd | 1.37de | 3.26abcd |
| 1.5 kg B ha⁻¹ at alternate years | 4.36a | 5.42abcd | 1.62cd | 3.57abc |
| 2.0 kg B ha⁻¹ at alternate years | 4.45a | 5.35abcd | 1.84abc | 3.67ab |
| 0.5 kg B ha⁻¹ every year         | 4.35a | 5.24abcd | 1.65b | 2.98abcd |
| 1.0 kg B ha⁻¹ every year         | 4.07a | 4.69d | 1.89ab | 3.10abcd |
| 1.5 kg B ha⁻¹ every year         | 4.38a | 5.30abcd | 1.95a | 3.72a |
| 2.0 kg B ha⁻¹ every year         | 4.14a | 5.59abc | 1.64bc | 3.38abcd |
| Control                          | 3.59b | 3.84e | 0.87g | 2.84d |
| LSD (p = 0.05)                   | 0.60  | 0.75  | 0.25  | 0.69 |

Values followed by different letters in columns are significantly different at \( P = 0.05 \) by LSD

The maximum amount of stover yield of rice was recorded under the application of B at 0.5 kg ha⁻¹ during alternate years. Application of B at 0.5 kg ha⁻¹ during alternate years in rice resulted in the improvement in the grain and straw yields to the tune of about 24% and 51%, respectively than no
application of B to this crop. Seed and stover yield of mustard was significantly improved when B applied at 0.5–2.0 kg ha\(^{-1}\) of the alternate year or each year. B applied at 1.5 kg ha\(^{-1}\) during each year significantly improved the seed and stover yield of mustard. This treatment resulted in increment of seed and stover yield to the tune of almost 124% and 31%, respectively, over no application of B. Concerning the system productivity of rice–mustard cropping system, application of B at 2.0 kg ha\(^{-1}\) in the alternate year resulted in the maximum yield being closely followed by the application of B at 1.5 kg ha\(^{-1}\) in every year (Fig. 1).

**B concentration and uptake**

The B concentration in rice grain and straw ranged from 11.83–23.03 mg kg\(^{-1}\) and 18.73–34.59 mg kg\(^{-1}\), respectively, while it (B concentration) was 13.07–27.20 mg kg\(^{-1}\) in mustard seed and 24.19–55.24 mg kg\(^{-1}\) in stover of mustard (Table 2). The maximum amounts of B concentration in both grain and straw of rice were recorded with the application of B at 2.0 kg ha\(^{-1}\) in every year. Significant improvement in the B content in rice grain was observed when the B applied in every year at 1.0–2.0 kg ha\(^{-1}\). B applied at 2.0 kg ha\(^{-1}\) in the first year also showed significant B concentration in rice grain. Application of B at 2.0 kg ha\(^{-1}\) in first year or every year resulted in the improvement of B concentration in rice grain about 75% – 78% compared to no application of B (control). In the case of B concentration in mustard seed, application of B at 1.5 kg ha\(^{-1}\) in the first year significantly improved B concentration than other treatments except for the application of B at 2.0 kg ha\(^{-1}\) in the first year. On the other hand, the application of B at 2.0 kg ha\(^{-1}\) in every year significantly improved B content in the stover of mustard. Concerning B content in mustard seed, it was observed that initial application of B at 1.5 or 2.0 kg ha\(^{-1}\) was better than other B application protocols, while, B application at 1.5 or 2.0 kg ha\(^{-1}\) in every year showed higher B content in mustard stover than other B application protocols.
Table 2
B concentration of rice and mustard as influenced by different B-application protocols

| Treatments                          | B concentration (mg kg$^{-1}$) |
|-------------------------------------|---------------------------------|
|                                     | Rice               | Mustard           |
|                                     | Grain  | Straw | Seed  | Stover |
| 0.5 kg B ha$^{-1}$ during first year| 11.83  | 18.73 | 17.09 | 29.02fg|
| 1.0 kg B ha$^{-1}$ during first year| 13.17fg| 20.00f| 21.27c| 33.32e |
| 1.5 kg B ha$^{-1}$ during first year| 17.63de| 25.30de|27.20a| 41.48c |
| 2.0 kg B ha$^{-1}$ during first year| 22.82ab| 29.32bc|25.02ab| 47.62b |
| 0.5 kg B ha$^{-1}$ at alternate years| 12.23g | 20.24f| 13.07f| 24.19h |
| 1.0 kg B ha$^{-1}$ at alternate years| 15.18ef| 24.17e| 14.38ef| 27.17g |
| 1.5 kg B ha$^{-1}$ at alternate years| 19.71cd| 28.23cd|20.97c| 36.64d |
| 2.0 kg B ha$^{-1}$ at alternate years| 20.10cd| 30.05bc|20.10c| 30.32f |
| 0.5 kg B ha$^{-1}$ every year       | 16.33e | 24.24e| 16.46de| 41.02c |
| 1.0 kg B ha$^{-1}$ every year       | 20.87abc| 32.80ab|20.08c| 47.62b |
| 1.5 kg B ha$^{-1}$ every year       | 20.32bc| 34.29a| 21.37c| 49.48b |
| 2.0 kg B ha$^{-1}$ every year       | 23.03a | 34.59a| 24.02b| 55.24a |
| Control                             | 13.02fg| 20.20f| 12.17f| 21.30i |
| LSD (p = 0.05)                      | 2.57   | 3.64 | 2.57  | 2.59 |

Values followed by different letters in columns are significantly different at $P = 0.05$ by LSD

In several B treatments, the B uptake by rice grain and straw was increased significantly than no application of B (Table 3). B uptake by rice grain was much higher when B application rate was increased i.e. >1.0 kg ha$^{-1}$ in the initial year or alternate years or every year. However, application of B at 1.0 kg ha$^{-1}$ in every year showed at par result with the application of B at 1.5–2.0 kg ha$^{-1}$ during the initial year or alternate years or every year. Concerning total B uptake by rice crop, the best result was estimated with B application at 2.0 kg ha$^{-1}$ in the first year. This treatment showed improvement in B uptake by the crop about 114% over no B application. All the B fertilization protocols resulted in significant improvement in B uptake by mustard seed than no B application. The highest amount of B uptake by mustard seed was recorded with the application of B at 1.5 kg ha$^{-1}$ in every year. B application at 1.5–2.0 kg ha$^{-1}$ in every year showed better uptake of B by the mustard crop. Concerning B uptake by stover almost similar trends
was recorded. The highest amount of total B uptake by mustard was recorded with the application of B at 2.0 kg ha$^{-1}$ in every year and this treatment was narrowly followed by application of B at 1.5 kg ha$^{-1}$ in every year. These two treatments showed more than 200% B uptake by the crop compared to no B application.

Table 3
B uptake by rice and mustard crop as influenced by different B-application protocols

| Treatments                        | **B uptake (mg kg$^{-1}$)** | **Rice** | **Straw** | **Total** | **Seed** | **Stover** | **Total** |
|-----------------------------------|----------------------------|----------|-----------|-----------|----------|------------|-----------|
| 0.5 kg B ha$^{-1}$ during first year |                           |         |           |           |          |            |           |
|                                   | Rice Grain                | 46.55e   | 88.82de   | 135.37    | 16.36e   | 86.52ef    | 102.88    |
|                                   | Mustard Total             | 152.47   | 211.59    | 364.06    |          |            |           |
| 1.0 kg B ha$^{-1}$ during first year |                           |         |           |           |          |            |           |
|                                   | Rice Grain                | 52.60de  | 99.87cde  | 152.47    | 18.59de  | 104.61d    | 123.21    |
|                                   | Mustard Total             | 202.57   | 271.2c    | 473.79    |          |            |           |
| 1.5 kg B ha$^{-1}$ during first year |                           |         |           |           |          |            |           |
|                                   | Rice Grain                | 74.65bc  | 127.92bc  | 202.57    | 27.12c   | 137.73bc   | 164.85    |
|                                   | Mustard Total             | 267.45   | 324.58b   | 592.03    |          |            |           |
| 2.0 kg B ha$^{-1}$ during first year |                           |         |           |           |          |            |           |
|                                   | Rice Grain                | 98.77a   | 168.68a   | 267.45    | 39.95c   | 152.08b    | 176.03    |
|                                   | Mustard Total             | 267.45   | 324.58b   | 632.03    |          |            |           |
| 0.5 kg B ha$^{-1}$ at alternate years |                         |         |           |           |          |            |           |
|                                   | Rice Grain                | 54.78de  | 98.18cde  | 152.96    | 15.57ef  | 73.04f     | 88.62     |
|                                   | Mustard Total             | 152.96   | 211.59    | 364.55    |          |            |           |
| 1.0 kg B ha$^{-1}$ at alternate years |                         |         |           |           |          |            |           |
|                                   | Rice Grain                | 65.08cde | 122.10bc  | 187.18    | 19.85de  | 89.08ef    | 108.93    |
|                                   | Mustard Total             | 211.59   | 271.2c    | 482.88    |          |            |           |
| 1.5 kg B ha$^{-1}$ at alternate years |                         |         |           |           |          |            |           |
|                                   | Rice Grain                | 86.13ab  | 125.46bc  | 211.59    | 33.96b   | 131.01bcd  | 164.96    |
|                                   | Mustard Total             | 211.59   | 271.2c    | 482.88    |          |            |           |
| 2.0 kg B ha$^{-1}$ at alternate years |                         |         |           |           |          |            |           |
|                                   | Rice Grain                | 89.38ab  | 100.88cde | 190.26    | 36.98ab  | 111.20cde  | 148.19    |
|                                   | Mustard Total             | 211.59   | 271.2c    | 482.88    |          |            |           |
| 0.5 kg B ha$^{-1}$ every year      |                           |         |           |           |          |            |           |
|                                   | Rice Grain                | 70.97bcd | 102.97cde | 173.94    | 27.15c   | 122.19bcd  | 149.35    |
|                                   | Mustard Total             | 190.26   | 271.2c    | 461.52    |          |            |           |
| 1.0 kg B ha$^{-1}$ every year      |                           |         |           |           |          |            |           |
|                                   | Rice Grain                | 84.98ab  | 154.02ab  | 239       | 37.90ab  | 147.92b    | 185.82    |
|                                   | Mustard Total             | 239      | 371.92b   | 611.87    |          |            |           |
| 1.5 kg B ha$^{-1}$ every year      |                           |         |           |           |          |            |           |
|                                   | Rice Grain                | 88.99ab  | 113.09cd  | 202.08    | 41.65a   | 183.86a    | 225.52    |
|                                   | Mustard Total             | 202.08   | 343.65b   | 545.73    |          |            |           |
| 2.0 kg B ha$^{-1}$ every year      |                           |         |           |           |          |            |           |
|                                   | Rice Grain                | 95.28a   | 124.25bc  | 219.53    | 39.37ab  | 186.92a    | 226.29    |
|                                   | Mustard Total             | 219.53   | 353.82b   | 573.35    |          |            |           |
| Control                           | Rice Grain                | 46.80e   | 77.99e    | 124.79    | 10.51f   | 60.71f     | 71.22     |
|                                   | Mustard Total             | 124.79   | 202.57    | 327.36    |          |            |           |
| LSD (p = 0.05)                    |                           | 19.84    | 33.53     | -         | 6.27     | 30.60      | -         |

Values followed by different letters in columns are significantly different at $P$ = 0.05 by LSD.
| Treatments | Readily soluble B (mg kg\(^{-1}\)) | Specifically adsorbed B (mg kg\(^{-1}\)) | Oxide bound B (mg kg\(^{-1}\)) | Organically bound B (mg kg\(^{-1}\)) | Residual B (mg kg\(^{-1}\)) | Total B (mg kg\(^{-1}\)) |
|------------|----------------------------------|---------------------------------|-------------------------------|-------------------------------|----------------------------|-------------------------|
| 0.5 kg B ha\(^{-1}\) during first year | 0.40(2.8)\(^g\) | 0.81(5.6)\(^{gh}\) | 2.33(16.1)\(^{cd}\) | 1.15(8.0)\(^{bc}\) | 9.77(67.6)\(^{b}\) | 14.46 |
| 1.0 kg B ha\(^{-1}\) during first year | 0.48(3.0)\(^g\) | 1.78(11.1)\(^{e}\) | 2.37(14.8)\(^{cd}\) | 1.18(7.4)\(^{bc}\) | 10.17(63.6)\(^{b}\) | 15.98 |
| 1.5 kg B ha\(^{-1}\) during first year | 0.51(2.8)\(^g\) | 1.84(10.1)\(^{de}\) | 2.48(13.6)\(^{c}\) | 1.26(6.9)\(^{bc}\) | 12.20(66.7)\(^{ab}\) | 18.29 |
| 2.0 kg B ha\(^{-1}\) during first year | 0.62(3.3)\(^{fg}\) | 1.98(10.5)\(^{d}\) | 2.53(13.4)\(^{bc}\) | 1.28(6.8)\(^{b}\) | 12.50(66.1)\(^{ab}\) | 18.91 |
| 0.5 kg B ha\(^{-1}\) at alternate years | 0.55(3.2)\(^{f}\) | 0.87(5.0)\(^{gh}\) | 2.82(16.3)\(^{abc}\) | 1.22(7.1)\(^{bc}\) | 11.80(68.4)\(^{ab}\) | 17.26 |
| 1.0 kg B ha\(^{-1}\) at alternate years | 0.92(4.3)\(^{e}\) | 0.94(4.4)\(^{g}\) | 3.22(15.1)\(^{abc}\) | 1.43(6.7)\(^{b}\) | 14.77(69.4)\(^{a}\) | 21.28 |
| 1.5 kg B ha\(^{-1}\) at alternate years | 1.23(6.2)\(^{d}\) | 2.19(11.0)\(^{c}\) | 2.95(14.8)\(^{abc}\) | 1.44(7.2)\(^{b}\) | 12.10(60.8)\(^{ab}\) | 19.91 |
| 2.0 kg B ha\(^{-1}\) at alternate years | 1.56(7.5)\(^{c}\) | 2.21(10.6)\(^{c}\) | 3.01(14.5)\(^{abc}\) | 1.48(7.1)\(^{b}\) | 12.56(60.3)\(^{ab}\) | 20.82 |
| 0.5 kg B ha\(^{-1}\) every year | 0.76(3.7)\(^{ef}\) | 1.24(6.1)\(^{f}\) | 3.47(17.0)\(^{ab}\) | 1.93(9.5)\(^{a}\) | 13.00(63.7)\(^{ab}\) | 20.40 |
| 1.0 kg B ha\(^{-1}\) every year | 1.63(7.2)\(^{bc}\) | 2.6(11.5)\(^{b}\) | 3.56(15.8)\(^{a}\) | 2.00(8.9)\(^{a}\) | 12.80(56.7)\(^{ab}\) | 22.59 |
| 1.5 kg B ha\(^{-1}\) every year | 1.83(7.8)\(^{ab}\) | 2.78(11.9)\(^{a}\) | 3.59(15.4)\(^{a}\) | 2.07(8.9)\(^{a}\) | 13.10(56.1)\(^{ab}\) | 23.37 |
| 2.0 kg B ha\(^{-1}\) every year | 1.92(8.0)\(^{a}\) | 2.81(11.7)\(^{a}\) | 3.64(15.2)\(^{a}\) | 2.09(8.7)\(^{a}\) | 13.46(56.3)\(^{ab}\) | 23.92 |
Treatments

| Treatments | Sequentially extracted B fractions (mg kg$^{-1}$) |
|------------|--------------------------------------------------|
|            | Readily soluble B | Specifically adsorbed B | Oxide bound B | Organically bound B | Residual B | Total B |
| Control    | 0.39(2.9)$^g$    | 0.71(5.3)$^h$          | 1.45(10.8)$^d$| 0.83(6.2)$^c$     | 10.10(74.9)$^b$| 13.48   |
| LSD (p = 0.05) | 0.23          | 0.16                  | 0.94         | 0.43               | 3.79      | -       |

Figure in parenthesis indicates the percentage of total; Values followed by different letters in columns are significantly different at $P = 0.05$ by LSD.

**B fractions**

The evaluation of the fractional soil B distribution showed that the percentage of different fractions to the total B extracted sequentially was specifically adsorbed B (4.4–11.9%), oxide bound B (10.8–17.0%), organically bound B (6.2–9.5%), and residual B (56.1%–74.9%) (Table 4). Most of the B protocols showed significantly higher specifically adsorbed B compared to no B application except B-application at 0.5 kg ha$^{-1}$ in the initial year or alternate years. Application of B at 1.5–2.0 kg ha$^{-1}$ resulted in the best attainment concerning specifically adsorbed B over all other B applications. Oxide bound B and organically bound B concentrations were improved in all the B-application protocols comparing no B application. However, the variations in these two B-fractions were very narrow when B applied in every year or alternate years. The range of readily soluble B varied from 0.39 mg kg$^{-1}$ – 1.92 mg kg$^{-1}$.

Application of B at 2.0 kg ha$^{-1}$ in every year showed the highest amount of readily soluble B and this treatment was at par with B-application at 1.5 kg ha$^{-1}$ in every year.

**Relationship between readily soluble B and grain yield of rice and seed yield of mustard**

Readily soluble B was related to both the yield of rice grain and as a mustard seed and B uptake by these two crops. Figure 2a and 2b represent the relationship between readily soluble B and yields of rice and mustard, respectively. $R^2$ values of Fig. 2a and 2b were 0.46 and 0.88, respectively. Thus, a strong relationship between soluble B and mustard yield was evaluated. However, the relationship between rice yield and soluble B was not so high as evaluated in the case of mustard yield. Likewise, Fig. 3a and 3b represent the relationship between soluble B with B uptake by rice and mustard, respectively. Here also the $R^2$ value was a higher increase of uptake of B by mustard than of in rice. Thus, a comparatively stronger relationship between soluble B and B-uptake by mustard was evaluated than that the relationship between B-uptake by rice and soluble B.

**Discussion**

Boron deficiency is the second most important micronutrient constraint of soils in India after that of zinc (Zn). Owing to this acute problem in Indian soil, considerable yield reduction in many crops was
previously reported [30], [31]. The availability of boron is mostly related to soil pH and is widely available at low pH but is frequently leached down from acid, sandy soils. Therefore, deficiency of boron prevails due to the low availability of the boron. Calcareous soils with low organic matter content are more prone to B deficiency [32]. The application of B increased rice yields might be due to its favourable effect on cell-dividing metabolic pathways [33]. Khan et al. [34] found that the application of B at 2 kg ha$^{-1}$ to rice along with recommended basal dose of N, P and K fertilizers resulted in the highest yield of the crop compared to other B application protocols. Remesh and Rani [35] reported the significant improvement of number of spikelet panicle$^{-1}$, grain weight and the number of filled grains panicle$^{-1}$ through the application of B at 1 kg ha$^{-1}$ than no application. Katyal and Singh [36] reported higher B-uptake by rice plant with soil applied B. They also reported that only less than 40% uptaken B was accumulated in rice-grain, and the remaining portion was accumulated in rice straws. Soil application of B at 10 kg ha$^{-1}$ enhanced the B content of rice grain [37]. They opined that the concentration of B in rice-grain and B-uptake by the grain were increased dramatically as B in the soil solution was increased through the B application in soil.

Boron (B) is an important micronutrient that contributes to mustard production and growth [31]. Many studies have shown that yields and yield attributes of the crop such as the number of silique plants$^{-1}$, 1000 seed weight etc. were significantly higher with the application of B [38],[39]. The B fractional data showed that the majority of B-fractions, except specifically adsorbed B, were improved with the application of B at 1.5–2.0 kg ha$^{-1}$ in alternate years or every year. The chances of losses of specifically adsorbed B from the upper soil might be higher compared with other fractions. The supply of specifically adsorbed B decreased due to the high pH level of calcareous soils [32]. B might be used during humification to bind to organic matter. In most agricultural lands, B-pool is highly correlated with humic colloids [40]. Gürel et al. [41] recorded the development of the B-buffer zone on organic-effect complexes, B-bound fractions leading to B-labile and making it less available for plant uptake. They also reported that the majority of B was present in the residual form (85–88%), followed by organically bound B (2.84–4.50%), adsorbed directly on the colloid soil surfaces (0.93–1.31%), oxide-bound B (7.27–8.31%), and readily soluble B which was the smallest ranging from 0.40–0.50% only.

**Conclusions**

From this experiment, it was observed that the application of B at 1.5 kg ha$^{-1}$ in every year or 2 kg ha$^{-1}$ in alternate years resulted in the highest yield of rice and mustard. Hence, the maximum system productivity of rice–mustard cropping system was also attained under these two B application protocols. Concerning B-uptake by the crop, application of 2 kg ha$^{-1}$ B in the initial year showed the best result, while, application of 1.5–2.0 kg ha$^{-1}$ B in every year resulted in the maximum B-uptake by the mustard crop. This protocol of B application (1.5–2.0 kg ha$^{-1}$ B in every year) also showed the maximum amount of readily soluble B. It was also observed that the relationships between readily soluble B with mustard yield as well as B-uptake by mustard were much stronger than rice yield as well as B-uptake by rice. So, from this experiment, it can be concluded that application of B at 2 kg ha$^{-1}$ in alternate years or 1.5 kg ha$^{-1}$ in
every year was better concerning the improvement of yield as well as soil available B under rice–mustard cropping system.

**Declarations**

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**Authors' contributions**

RL, SKS, BP, VK and DN were involved in conception, design, acquisition, analysis and interpretation of the data, and wrote the original draft. ESD, AOA, MMH and AH were involved in conception of the study, and subsequent drafting. All authors worked on and approved the final manuscript for submission to the journal.

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**Availability of data and materials**

All data recorded and analysis during this study are available in Tables and Figures

**Declarations:**

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Competing interests**

All authors declare no conflict of interest.

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Figures

![Figure 1](image_url)

**Figure 1**

System productivity of the rice–mustard cropping system as influenced by different B-fertilization protocols. Note: T1: 0.5 kg B ha⁻¹ during the first year; T2: 1.0 kg B ha⁻¹ during the first year; T3: 1.5 kg B ha⁻¹ during the first year; T4: 2.0 kg B ha⁻¹ during the first year; T5: 0.5 kg B ha⁻¹ during alternate years;
T6: 1.0 kg B ha\(^{-1}\) during alternate years; T7: 1.5 kg B ha\(^{-1}\) during alternate years; T8: 2.0 kg B ha\(^{-1}\) during alternate years; T9: 0.5 kg B ha\(^{-1}\) during every year; T10: 1.0 kg B ha\(^{-1}\) during every year; T11: 1.5 kg B ha\(^{-1}\) during every year; T12: 2.0 kg B ha\(^{-1}\) during every year; T12: Control (no-application of B); REY denotes rice equivalent yield; lines above the bar diagram depict the standard deviation (n = 3).

Figure 2

(a) Relationship between soil readily soluble B with grain yield of rice; and (b) seed yield of mustard.

\[ y = -0.7145x^2 + 1.778x + 3.3441 \]
\[ R^2 = 0.4578 \]

\[ y = -0.6038x^2 + 2.0052x + 0.1625 \]
\[ R^2 = 0.882 \]
Figure 3

(a) Relationship between readily soluble B with B uptake by rice; and (b) B uptake by mustard.