



Enhancing drought tolerance in Indian mustard (*Brassica juncea*) through microbial inoculants under varying irrigation regimes

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ABSTRACT

A field experiment was conducted during the winter (*rabi*) seasons of 2020–21, 2021–22 and 2022–23 at College of Agriculture, Sri Karan Narendra Agriculture University, Jobner, Rajasthan to identify the appropriate bacterial strains and irrigation levels to enhance drought tolerance and productivity in Indian mustard [*Brassica juncea* (L.) Czern.]. The experiment was laid out in a split plot design assigning irrigation treatments (no irrigation, 50% deficit irrigation and normal level of irrigation) in main plots and microbes (MRD-17, MKS-6, Biophos and Biophos⁺, CRIDAMI-I, CRIDA MI-II and control) in sub plots. Results demonstrated that normal level of irrigation (2 irrigations) yielded superior outcomes in terms of plant height, dry matter accumulation, yield attributes, viz. seed yield (1542 kg/ha), stover yield (3677 kg/ha), relative water content (70.72%), protein content (18.1%), oil content (38.7%), water use efficiency (7.50 kg/ha-mm), net returns (₹52529/ha) and B:C ratio (2.07). This was significantly higher compared to 50% deficit irrigation and no irrigation treatments. Among microbial inoculants, CRIDA MI-II exhibited the highest impact on growth and yield attributes, viz. seed yield (1312 kg/ha), stover yield (3262 kg/ha), protein content (17.2%), water use efficiency (5.88 kg/ha-mm), net returns (₹42294/ha) and B:C ratio (1.72). Notably, it was statistically comparable to the effects of CRIDA MI-I and Biophos and Biophos⁺ across most parameters. The comprehensive results of this study advocated the strategic implementation of both optimal irrigation practices and microbial inoculants as a sustainable approach to enhance productivity and drought tolerance in Indian mustard, particularly in the arid and semi-arid regions of Rajasthan.

Keywords: Irrigation, Microbes, Mustard, Relative water content, Yield

Indian mustard [*Brassica juncea* (L.) Czern.] is an important oilseed globally, particularly in India, where it is predominantly grown as an edible oilseed crop. Mustard seeds exhibit a varying oil content ranging from 28.6 to 45.7%. Rajasthan holds the first position in both the cultivated area and production of mustard in India (Anonymous 2022). Although mustard doesn't have very high-water requirement, it has to have a proper water balance to reach its maximum potential, especially during crucial growth stages when it is susceptible to moisture stress. For plants to flourish, the root zone must have the ideal moisture content, reducing stress at vulnerable times (Bodner *et al.* 2015). Timely irrigation scheduling and effective conservation practices are essential for achieving this balance. Furthermore, adopting suitable irrigation schedules can positively impact both crop yield and quality (Rai *et al.* 2017). The judicious application of irrigation

water is crucial, aligning with the specific needs of crops. Due to insufficient and unpredictable rainfall during *rabi* season, mustard cultivation necessitates supplementary irrigation for optimal growth and development. Without adequate irrigation, the crop is susceptible to water stress, potentially leading to a decline in yield.

Recent research suggests that microorganisms can serve as a beneficial and environmental friendly method against drought stress, offering a cost-effective approach. Meena *et al.* (2017) emphasized that various bacterial genera, including *Bacillus*, *Pseudomonas*, *Rhizobium*, *Paenibacillus*, *Burkholderia*, *Achromobacter*, *Azospirillum*, *Enterobacter* and others contribute to drought tolerance in host plants, enhancing their growth. Over the past two decades, numerous reports have emerged, showcasing the potential of such microbes in inducing tolerance against drought stress. The potential of plant growth-promoting rhizobacteria (PGPR) from harsh environments to shield plants from the damaging effects of drought stress was highlighted by Kavamura *et al.* (2013). This could ultimately increase crop yield in arid or semi-arid locations. Therefore, the present study aimed to identify appropriate bacterial

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strains and irrigation levels to enhance drought tolerance and productivity in Indian mustard.

MATERIALS AND METHODS

A field experiment was conducted during the winter (*rabi*) seasons of 2020–21, 2021–22, and 2022–23, at Agronomy Farm, Sri Karan Narendra College of Agriculture, Jobner (26°05' N; 75°28' E; altitude of 427 m amsl), Rajasthan. The climate is distinctly semi-arid, characterized by notable temperature variations throughout the year. Summers are marked by high temperatures, occasionally reaching up to 48°C, while winters experience a significant temperature drop, with cold lows reaching as far as -1.0°C. The soil of experimental field was classified as loamy sand having low organic carbon content (0.22%), 139.8 kg/ha of available nitrogen, 16.9 kg/ha of available phosphorus and a medium level of available potassium (151.6 kg/ha). The experimental was conducted in a split-plot design, encompassing 18 distinct experimental treatments replicated thrice. The main plots were assigned with 3 different irrigation levels, viz. no irrigation; 50% deficit irrigation; and normal level of irrigation while the sub-plots accommodated 6 distinct microbial inoculants, viz. MRD-17; MKS-6; Biophos and Biophos⁺; CRIDA MI-I; CRIDA MI-II and Control. Under normal level of irrigation, two irrigations were applied, one at pre-flowering and other at silique development stage, while under 50% deficit irrigation treatment, only one irrigation was applied at pre-flowering stage. MRD-17 and MKS-6 are strains of *Bacillus* spp., which are osmotolerant rhizobacteria. Biophos and Biophos⁺ are liquid formulations of phosphorus solubilizing bacteria containing *Paenibacillus* spp. and *Kluyvera* spp., respectively. CRIDA MI-I is liquid formulation of *Pseudomonas putida* + *Paenibacillus favisporus* strain B30 and CRIDA MI-II is a liquid formulation of *Pseudomonas putida* P45 + *Bacillus amyloliquefaciens* B17. The seeds of mustard were inoculated with these microbial inoculants prior to sowing. The mustard crop under experimentation received a uniform dose of 60 kg/ha of nitrogen (N) and 30 kg/ha of phosphorus (P) through urea and diammonium phosphate (DAP), respectively. At the time of sowing, half of the nitrogen dose, along with the complete phosphorus dose, was applied as a basal treatment. The remaining nitrogen was then top-dressed at 40 days after the sowing (DAS). Mustard variety Laxmi seeds were sown at a rate of 5 kg/ha in the experimental setup. The sowing process involved placing the seeds in rows with a 30 cm spacing behind the plough. Following the seed placement in the furrow, the ridges were disassembled to ensure adequate soil coverage for the seeds. Prior to sowing, a standard irrigation of 40 mm was administered to facilitate germination. Subsequent irrigations were then carried out with measured water quantities, tailored to the specific irrigation treatments.

The mustard crop was harvested at the maturity stage from the designated area within each individual plot. The harvested material was then bundled separately, tagged,

and left for sun drying in the field. Following thorough sun drying, the bundles were weighed to document the biological yield. Manual threshing was conducted by gently beating the dried bundles of each plot with a wooden stick, followed by winnowing. After the cleaning process, the seed yield per plot was recorded and converted into kg/ha. Stover yield was determined by subtracting the seed yield from the previously recorded biological yield. The Arnon (1949) methodology was used to determine the amount of chlorophyll at 80 DAS. 50 mg of fresh leaf material were used in this procedure, and 80% acetone was used to homogenize the samples. After that, an aliquot was taken out and centrifuged at 2000 rpm for 10 min. A 10 ml final volume adjustment was made. Thereafter, a Spectronic-20 spectrophotometer was used to measure the absorbance of the clear liquid supernatant.

$$\text{Total chlorophyll} = \frac{A_{(652)} \times 29^* \times \text{Total volume (ml)}}{\alpha \times 1000 \times \text{weight of sample (g)}}$$

where α , path length (1 cm); 29* is constant.

Relative water content (RWC) in leaf samples was determined using the procedure described by Barrs and Weatherley (1962). A petri dish containing double-distilled water was placed with 1 g of completely opened fresh leaf sample (FW), and the assembly was left at room temperature for 4 h. The leaf samples were then removed, the excess water was gently blotted off, and the turgid weight (TW) was recorded. The leaf samples were then dried in an oven for the entire night at 65°C, and the resulting dry weight (DW) was noted. Relative water content (RWC) was calculated as:

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

For a thorough assessment, the economic considerations of the various treatments were analyzed by considering the input costs and the revenue generated from the output at the current market prices. The water-use efficiency (WUE) was computed (Viets 1966) and expressed as kg/ha-mm:

$$\text{WUE} = \frac{\text{Seed yield (kg /ha)}}{\text{Actual evapo-transpiration (mm)}}$$

Protein content in the seed was determined as:

$$\text{Protein (\%)} = \text{Nitrogen} \times 6.25$$

The oil content in mustard seed was assessed using a Soxhlet apparatus with petroleum ether (60–80°C) as an extractant, following the method outlined in the A.O.A.C. (1960). The experimental data gathered for growth, yield, and quality characteristics underwent statistical analysis using the 'Analysis of variance' method proposed by Fisher (1950). To assess the significance of variations among treatment effects in split-plot design experiments, the 'F' test was employed (Panse and Sukhatme 1985).

RESULTS AND DISCUSSION

Growth parameters: The pooled data of 3-years of the present experiment revealed that application of normal

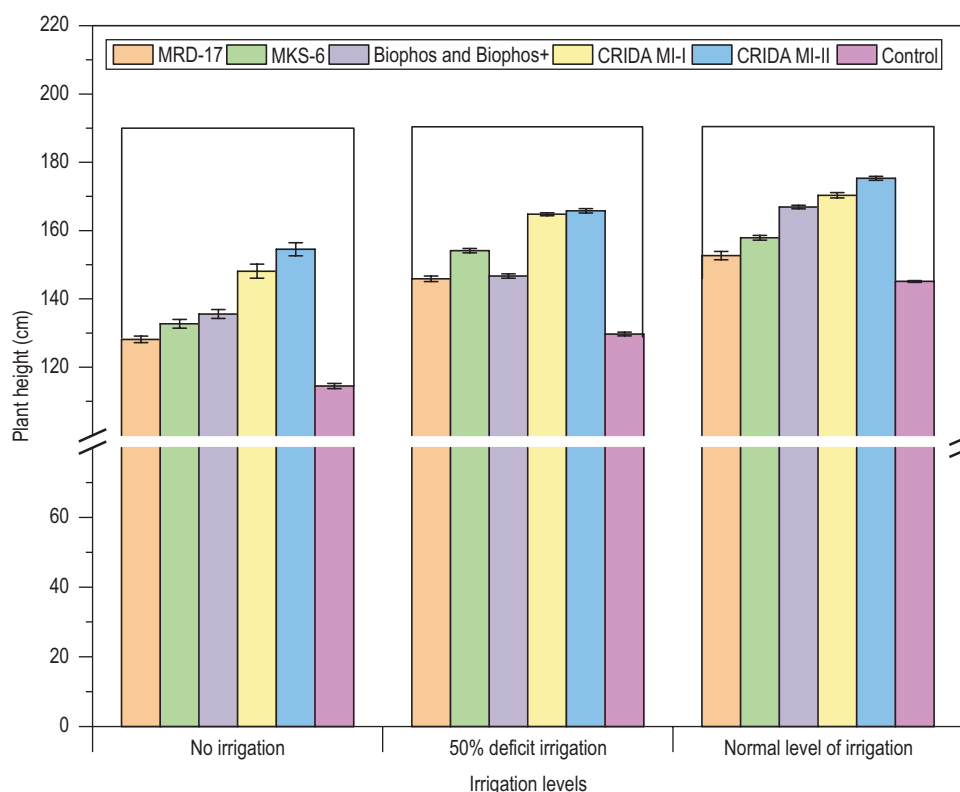


Fig. 1 Effect of irrigation levels and microbial inoculants on plant height of Indian mustard.

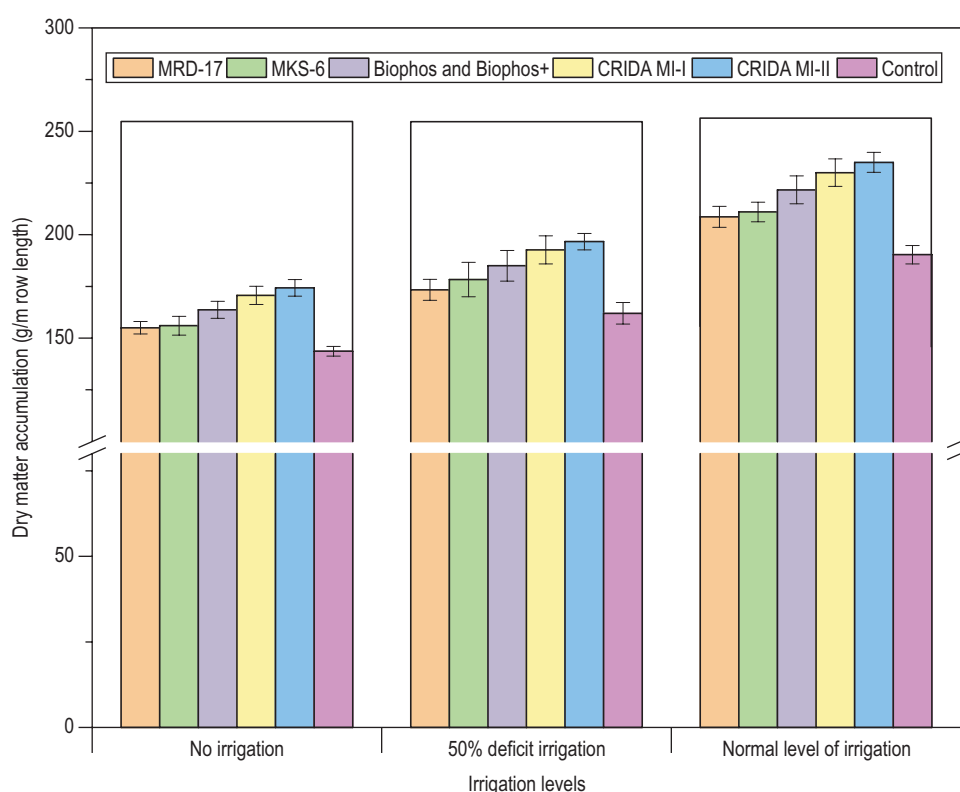


Fig. 2 Effect of irrigation levels and microbial inoculants on dry matter accumulation of Indian mustard.

level of irrigation (two irrigations) recorded the maximum plant height (161.4 cm) and dry matter accumulation (216.1 g/m row length) of Indian mustard at harvest, which was

significantly higher over 50% deficit irrigation and no irrigation treatment (Fig. 1 and 2). The administration of a regular level of irrigation over control (no irrigation) resulted in a 19.0 and 34.5% increase in plant height and dry matter output of mustard, respectively. It is widely acknowledged that maintaining adequate soil moisture through irrigation promotes increased development of green tissue area, leading to enhanced photosynthetic assimilation (Bharti *et al.* 2007). Consequently, this improvement in plant growth resulted in higher accumulation of total dry matter. The current findings in mustard are consistent with those of Mishra *et al.* (2019).

Among various microbial inoculants, the most noteworthy results in terms of plant height and dry matter were achieved through the application of CRIDA MI-II. Although statistically equivalent to CRIDA MI-I, Biophos, and Biophos+, the CRIDA MI-II demonstrated a substantial advantage over MRD-17, MKS-6, and the control. Additionally, in comparison to the uninoculated control, the MRD-17 and MKS-6 inoculation also enhanced the plant height and dry matter of mustard. The plant height and dry matter accumulation were increased by 27.0 and 22.2% over control through CRIDA MI-II inoculation. Rhizobacteria positively influence plant height and dry matter accumulation by producing growth-promoting substances, enhancing

nutrient uptake, and improving stress tolerance. These beneficial bacteria contribute to increased structural development, nutrient assimilation, and overall plant health,

Table 1 Effect of irrigation levels and microbial inoculants on yield attributes and yield of Indian mustard (Pooled data of 3-years)

Treatment	Silique/plant	Seeds/silique	Test weight (g)	Seed yield (kg/ha)	Stover yield (kg/ha)
Irrigation level					
M ₁ , No irrigation	143.6	10.7	3.64	893	2195
M ₂ , 50% deficit irrigation	159.8	12.4	3.94	1216	3073
M ₃ , Normal level of irrigation	177.2	13.2	4.19	1542	3677
S.Em±	3.06	0.39	0.06	34.6	55.7
CD (<i>P</i> =0.05)	9.87	0.79	0.19	81.7	218
Microbial inoculant					
S ₁ , MRD-17	155.0	11.6	3.81	1187	2879
S ₂ , MKS-6	160.2	12.0	3.88	1224	2951
S ₃ , Biophos and Biophos ⁺	162.8	12.2	3.97	1235	3031
S ₄ , CRIDA MI-I	168.2	12.8	4.06	1264	3193
S ₅ , CRIDA MI-II	172.9	13.0	4.12	1312	3262
S ₆ , Control (No culture)	142.0	10.9	3.70	1081	2575
S.Em±	2.56	0.25	0.05	23.8	99.6
CD (<i>P</i> =0.05)	8.95	0.56	0.16	74.6	287

promoting higher plant productivity. Nivetha *et al.* (2020) also reported similar results in mustard.

Yield attributes and yield: The application of normal irrigation levels resulted in the optimum enhancement of various yield parameters in mustard, including silique/plant (177.2), seeds/silique (13.2), test weight (4.19 g), and both seed (1542 kg/ha) and stover yield (3677 kg/ha) (Table 1). These results were significantly superior to both 50% deficit irrigation and the control. Notably, normal irrigation levels exhibited a considerable increase of 72.6% in mustard seed yield compared to 50% deficit irrigation and no irrigation treatment. Additionally, the application of 50% deficit irrigation demonstrated a significant improvement in mustard yield and associated attributes compared to the non-irrigated control. This observation is likely attributed to the correlation between seed yield and both the dry matter content and yield attributes of the plant, which exhibited a significant increase with higher irrigation frequency. The occurrence can be attributed to increased soil moisture availability in the mustard crop's rhizosphere, which encourages increased production of photosynthates and improved transfer of photosynthates to reproductive structures. The results are in close conformity with the findings of Yadav *et al.* (2021) and Maurya *et al.* (2022).

Microbial inoculation in mustard demonstrated a substantial improvement in both yield attributes and overall yield compared to the control (no culture). Among the inoculants, CRIDA MI-II exhibited the most pronounced effects, resulting in the highest values for silique/plant (172.9), seeds/silique (13.0), test weight (4.12 g), as well as seed (1312 kg/ha) and stover yield (3262 kg/ha). Notably, CRIDA MI-I, Biophos, and Biophos⁺ demonstrated comparable effects, showing statistically similar impacts on

these parameters. Application of CRIDA MI-II increased the seed and stover yield of mustard by 23.6 and 26.6%, respectively over un-inoculated control. Additionally, the inoculation of MKS-6 and MRD-17 also led to a significant increase in yield attributes and overall mustard yield compared to the non-inoculated control. Microbial inoculants contribute to increased crop yield under drought stress by enhancing water and nutrient uptake, promoting root development, and triggering stress-tolerance mechanisms. These beneficial microorganisms mitigate the adverse effects of drought by improving the plant's ability to withstand water scarcity. The resulting improvement in plant resilience and resource utilization ultimately leads to higher yields in drought-challenged environments. The current findings are consistent with those of Asha *et al.* (2021).

Physiological attributes and quality: The determination of chlorophyll content in plants serves as a crucial parameter for assessing photosynthetic efficiency, particularly in the context of drought stress. In the present experiment, the pooled data of 3-years showed that application of normal level of irrigation recorded maximum chlorophyll content (3.36 mg/g) of mustard leaves over 50% deficit irrigation and unirrigated control (Table 2). The minimum chlorophyll content was observed under no-irrigation treatment (2.12 mg/g). Differential irrigation levels significantly influence chlorophyll content in crops, with optimal irrigation positively impacting photosynthetic performance, while water deficit conditions often lead to a decline in chlorophyll levels, indicating potential stress on the photosynthetic apparatus. Similarly, the relative water content (RWC) of mustard leaves exhibited a progressive increase corresponding to elevated irrigation levels, reaching its peak

Table 2 Effect of irrigation levels and microbial inoculants on physiological attributes and quality parameters of Indian mustard (Pooled data of 3-years)

Treatment	Chlorophyll content (mg/g)	Relative water content (%)	Protein content (%)	Oil content (%)
<i>Irrigation level</i>				
M ₁ , No irrigation	2.12	55.56	15.9	36.7
M ₂ , 50% deficit irrigation	2.73	64.50	17.0	37.5
M ₃ , Normal level of irrigation	3.36	70.72	18.1	38.7
S.E.m±	0.08	1.64	0.18	0.38
CD (P=0.05)	0.22	4.88	0.51	1.39
<i>Microbial inoculant</i>				
S ₁ , MRD-17	2.57	62.89	16.8	37.7
S ₂ , MKS-6	2.66	63.78	17.0	37.6
S ₃ , Biophos and Biophos ⁺	2.84	64.00	17.4	37.8
S ₄ , CRIDA MI-I	2.95	64.56	17.2	37.5
S ₅ , CRIDA MI-II	3.01	65.33	17.2	38.1
S ₆ , Control (no culture)	2.37	61.00	16.4	36.9
S.E.m±	0.08	1.88	0.23	0.53
CD (P=0.05)	0.24	NS	0.67	NS

under normal irrigation conditions (70.72%). Conversely, the lowest RWC was observed in the unirrigated control (55.56%), indicating a direct correlation between irrigation levels and the water status of mustard leaves. The increase in RWC with higher irrigation frequency is primarily due to the improved water availability in the plant tissues. Adequate irrigation ensures a continuous supply of water to the plant, preventing water stress and desiccation. This sustained water availability enhances turgor pressure within the plant cells, maintaining cell hydration and leading to a higher RWC. Further, the protein and oil content in mustard demonstrated a notable increase under optimal irrigation conditions, attaining its highest level with normal irrigation (18.1% and 38.7%, respectively). In contrast, the lowest protein content was registered in the unirrigated control, highlighting the direct influence of irrigation levels on mustard plant protein synthesis. The outcomes of Singh *et al.* (2021) are consistent with these findings.

Application of microbial inoculations also brought about significant increase in chlorophyll content of mustard over control. The highest chlorophyll content was recorded under inoculation of CRIDA MI-II (3.01 mg/g) followed by CRIDA MI-I (2.95 mg/g) and both these treatments were statistically at par with Biophos and Biophos⁺ (2.84 mg/g). These beneficial bacteria enhance nutrient availability, stimulate root development, and produce growth-promoting substances, all of which contribute to improved

photosynthetic performance. The increased chlorophyll content is a reflection of enhanced photosynthetic efficiency, leading to improved plant growth and potentially higher crop yields. Similar findings have also been reported by Asha *et al.* (2021). In the current investigation, microbial inoculation did not, however, show any appreciable impact on the relative water content and oil content of mustard. Further, the application of Biophos and Biophos⁺ demonstrated a significant increase in protein content in mustard seeds, attaining levels statistically comparable to those observed with CRIDA MI-II and CRIDA MI-I. However, these results were notably superior to the effects observed with MKS-6, MRD-17, and the control. This underscores the efficacy of Biophos and Biophos⁺ in enhancing protein synthesis in mustard seeds, positioning it as an effective bioinoculant for crop improvement. Similar findings have also been reported by Asha *et al.* (2021).

Water use efficiency and economics: The highest WUE was achieved under normal irrigation levels (7.50 kg/ha-mm), exhibiting a statistically significant increase compared to both 50% deficit irrigation (5.14 kg/ha-mm) and the control (3.90 kg/ha-mm). The augmentation of irrigation levels has a direct and positive effect on the increase in WUE of crops. As irrigation frequency rises, plants exhibit enhanced physiological processes, optimizing water utilization. This leads to improved WUE, where crops efficiently convert water resources into biomass and yield. The positive correlation between increasing irrigation levels and heightened WUE underscores the significance of appropriate water management practices in promoting sustainable and efficient crop production. Similar results were also reported by Singh *et al.* (2017). In terms of economic returns, the application of normal irrigation levels resulted in the highest gross return (₹77857/ha), net return (₹55529/ha), and benefit-cost (B:C) (2.07) ratio in mustard. This increase in economics was significantly superior to both 50% deficit irrigation and the unirrigated control. These findings underscore the economic advantages associated with maintaining optimal irrigation practices, highlighting the potential for increased profitability and overall economic viability in mustard. The outcomes closely align with the findings reported by Shivran *et al.* (2018) in mustard.

The application of microbial inoculants resulted in a substantial improvement in the WUE of mustard compared to the control (Table 3). Among the inoculations, CRIDA MI-II exhibited the highest WUE (5.88 kg/ha-mm), statistically equivalent to CRIDA MI-I (5.74 kg/ha-mm), Biophos, and Biophos⁺ (5.56 kg/ha-mm). In contrast, the uninoculated control demonstrated the lowest WUE (5.08 kg/ha-mm). Moreover, MKS-6 and MRD-17 also enhanced WUE of mustard over control. Plant growth-promoting rhizobacteria (PGPRs) have been found to positively impact the water use efficiency (WUE) of mustard crops. Application of PGPRs enhances nutrient uptake, root development and stress tolerance, contributing to improved water utilization by the plants. The resulting increase in WUE suggests the potential of PGPRs in enhancing water conservation and

Table 3 Effect of irrigation levels and microbial inoculants on economics and WUE of Indian mustard (Pooled data of 3-years)

Treatment	Gross returns (₹/ha)	Net returns (₹/ha)	Benefit : Cost ratio	WUE (kg/ha-mm)
<i>Irrigation level</i>				
M ₁ , No irrigation	45427	22411	0.98	3.90
M ₂ , 50% deficit irrigation	62218	38001	1.57	5.14
M ₃ , Normal level of irrigation	77857	52529	2.07	7.50
S.E.m±	1458	1458	0.06	0.12
CD (P=0.05)	3899	3899	0.16	0.49
<i>Microbial inoculant</i>				
S ₁ , MRD-17	60161	36011	1.47	5.39
S ₂ , MKS-6	61846	37696	1.54	5.43
S ₃ , Biophos and Biophos ⁺	62542	38209	1.55	5.56
S ₄ , CRIDA MI-I	64826	40543	1.65	5.74
S ₅ , CRIDA MI-II	66578	42294	1.72	5.88
S ₆ , Control (no culture)	55053	31131	1.28	5.08
S.E.m±	1234	1234	0.05	0.14
CD (P=0.05)	3321	3321	0.12	0.40

WUE, Water use efficiency.

optimizing water resource utilization in mustard. This is consistent with other research indicating that rhizobacteria may improve plants' water-use efficiency, especially in the presence of drought stress (Mayak *et al.* 2004). From an economic standpoint, the application of CRIDA MI-II yielded the highest net return of ₹42294/ha with a commendable B:C ratio of 1.72. This economic outcome was statistically comparable to the performance of CRIDA MI-I (₹40543/ha and 1.65). Notably, these results were significantly superior to the net returns obtained under MRD-17, MKS-6, and the control treatment, highlighting the economic viability of these microbial inoculants in mustard. These findings underscore the positive economic implications of incorporating microbial inoculants in mustard crop, indicating a potential for enhanced profitability and resource efficiency.

Based on the findings, it is recommended to adopt a 2-irrigation strategy for optimal productivity of mustard, as it consistently outperformed 50% deficit irrigation and no irrigation treatments. Additionally, incorporating microbial inoculants, especially CRIDA MI-II, CRIDA MI-I, Biophos, and Biophos⁺, is advised to enhance drought tolerance and overall yield. This integrated approach holds promise for sustainable mustard farming in the arid and semi-arid regions of Rajasthan, offering a practical solution to address the challenges posed by drought stress.

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