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The Impact of Nitrogen-Fixing Bacteria, Iron, and Zinc Foliar Application on Dry Land Yellow Mustard (*Brassica juncea*) Grain and Oil Production

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Abstract

The study, conducted at the Research Farm of the College of Agriculture, University of Tabriz in 2021, focused on the effects of various nitrogen-fixing bacterial isolates, biofertilizers containing nitrogen and phosphorus, as well as iron and zinc foliar applications on mustard growth under rainfed conditions. The results indicated that biofertilizers, whether used alone or in combination with chemical fertilizers, produced comparable grain and oil outputs compared to chemical fertilizers alone. Additionally, the application of iron and zinc through foliar spraying significantly enhanced both grain and oil production. These findings suggest that integrating nitrogen-fixing bacteria and biofertilizers could reduce reliance on chemical nitrogenous fertilizers, leading to decreased production expenses, improved product quality, and minimized environmental impact. This study highlights the potential for sustainable agricultural practices in dry land farming as a viable alternative to traditional chemical-intensive methods. Substituting chemical nitrogenous fertilizers with nitrogen-fixing bacteria or biofertilizers could result in cost savings in mustard grain and oil production while promoting environmental sustainability.

Keywords

Nitrogen Fixing Bacteria, Yellow Mustard, Dry Land Farming, Iron, Zinc, Foliar Application

1. Introduction

Dryland ecosystems have a rich history across all continents and are significant for hosting diverse flora with unique plant lineages found in various regions globally [1].

Indian or yellow mustard (*Brassica juncea* L.), belonging to the Brassicaceae family, stands out as one of the leading oilseed crops worldwide, currently ranked third in terms of both production volume and cultivated area. Rapeseed and mustard are the main oilseed crops cultivated in India during the rabi season, with oil quantity and quality being crucial factors for Indian mustard, primarily influenced by mineral fertilization [2] [3].

The productivity of mustard is often constrained by suboptimal fertilizer application on marginal lands under rain-fed conditions. Both the quantity and quality of mustard oil are significantly impacted by mineral nutrition. Effective nutrient management is essential for boosting crop yield, with fertilizers playing a key role in enhancing agricultural productivity through soil fertility improvement. However, excessive use of chemical fertilizers can result in environmental pollution and soil degradation, highlighting the necessity to embrace sustainable agricultural practices that reduce reliance on toxic chemicals [4].

To minimize pollutants in agro-ecosystems, it is recommended to avoid synthetic chemical pesticides and fertilizers during agricultural processes [5].

Biofertilizers containing nitrogen-fixing bacteria present an eco-friendly alternative that can enhance soil fertility and crop productivity while reducing dependency on chemical inputs. Nitrogen-fixing bacteria convert atmospheric nitrogen into ammonia, benefiting plant metabolic processes [6] [7].

Yellow mustard is particularly sensitive to nitrogen, zinc, and iron deficiencies, which can hamper growth and productivity. Zinc and iron are vital micronutrients essential for enzyme activation, pod setting, seed formation, and oil synthesis in mustard seeds [8]. Indian mustard's sensitivity to zinc (Zn), iron (Fe), and nitrogen (N) deficiencies can lead to reduced productivity due to small leaves, chlorosis, and dwarfing. Zinc and iron are crucial micronutrients required in trace amounts by humans, animals, and plants as structural components of enzymes necessary for several biological functions in mustard seeds [9] [10].

This study aims to assess the impact of nitrogen-fixing bacteria isolates along with foliar applications of iron and zinc on yellow mustard production under dry land conditions with the objective of identifying sustainable practices that reduce reliance on chemical nitrogen fertilizers while maintaining or enhancing crop yield and quality.

2. Materials and Methods

2.1. Experimental Site and Treatments

Two field experiments conducted in northwest Iran at the Research Farm of College of Agriculture, University of Tabriz, in 2021. The soil composition of the research area was sandy loam with specific characteristics including pH 7.24, EC

0.78 dS/m, OM 0.89%, $CaCO_3$ 14.8%, N 0.13%, P 11.62 mg/kg, and K 502.2 mg/kg. This region is categorized as semi-arid with an average annual rainfall of 250 mm and a mean annual temperature of 10˚C.

The Sadegh cultivar of yellow mustard from Dryland Institute of Iran was planted in randomized complete blocks with three replicates on April 20, 2021, using a seeding rate of 80 seeds/m^2 in plots consisting of six rows each measuring 3 m in length and spaced at intervals of 25 cm. Irrigation was applied once after sowing, and precipitation levels during the growing season were recorded for April (15.3 mm), May (22.2 mm), June (15.8 mm), July (14.9 mm), and August (0 mm).

The experimental treatments included seven options: control (Chemical fertilizer at rates of 250 kg/ha urea and 150 kg/ha phosphorus based on soil analysis), bacterial isolates belonging to Enterobacteria-3MDP-1, -3MDP-6, -2MDP-10, combinations of bacterial isolates with chemical fertilizer at reduced rates (50%) for the first experiment; while for the second experiment treatments comprised control group as well as biofertilizers Ntrozist for nitrogen-fixing bacteria, Phophozist for phosphorous, combination treatment Nitozist + Phosphozist, Iron foliar application, Zinc foliar application, and Iron+ Zinc foliar application.

Nitrogen-fixing bacterial isolates were acquired from the Soil Microbiology Lab at Soil Science Department of University of Tabriz and used as seed inoculants during sowing process. Additionally, Nitrozist (containing Enterobacteria) and Phosphozist (containing Phosphorus-solubilizing bacteria) biofertilizers were obtained from Kesht Kar Gostar Nozhan Co., applied through fertigation at a rate of 5 liters/ha during stem elongation phase. For iron and zinc supplementation via foliar application during flowering stage FeSo4 and ZnSo4 0.1% w/v solutions were utilized respectively.

2.2. Sampling and Data Collection

Sampling involved harvesting seeds post physiological maturity from a designated area within central rows in August 2021 to calculate grain yield and biological yield per square meter by drying plants in an oven at 75˚C for 48 h followed by weight determination; further evaluation included components such as thousand grains weight and number per plant from randomly selected samples taken from central rows to determine grain yield components.

10 grams of each seed sample was placed in the thimble and inserted into the center of the extractor, which was then heated to a temperature range of 40˚C - 60˚C. As the solvent boiled, vapor traveled up the vertical tube into the condenser located at the top. The resulting liquid condensate dripped into the filter paper thimble containing the solid sample for extraction. The extract permeated through the thimble's pores, filling the siphon tube and flowing back down into the round bottom flask. This process continued for 30 minutes before removal from the tube, followed by drying in an oven, cooling in desiccators, and reweighing to determine oil extraction levels.

Data analysis was conducted using SPSS software based on experimental design criteria. Means of individual traits were statistically compared using Duncan multiple range tests at a significance level of $P \le 0.05$.

3. Results

3.1. Morphological Characteristics

In terms of morphological characteristics, plant height showed significant variation among different fertilizer treatments in two experiments. For instance, mustard plants treated with 3MDP-1 exhibited greater height compared to other treatments in the first experiment; however, differences with some other treatments were not statistically significant. Conversely, mustard plants subjected to 2MDPI-10 treatment were notably shorter than those under alternative conditions. The number of lateral branches varied across different treatments as well. For instance, mustard plants treated with 3MDPI-1 had more lateral branches compared to other plots, while those exposed to 3MDPI-6 treatment exhibited lower numbers of lateral branches (Table 1).

Additionally, plants treated with iron and zinc foliar application displayed increased height and above-ground dry weight relative to other treatments in another experiment (Table 2).

Table 1. The impact of nitrogen fixing bacteria isolates and chemical fertilizer on yellow mustard performance.

Treatments	Plant height (cm)	Number of branches	Number of pods	Grain yield ($g \cdot m^{-2}$)	1000 seeds weight (g)	Oil yield ($g \cdot m^{-2}$)
100% Chemical fertilizer	65.00bc*	9.51ab	113.6a	263a	3.81a	141.9b
3MDPI-1	77.52a	10.94a	112.45a	266a	3.59a	88.3c
3MDPI-6	71.71ab	4.68d	71.1b	215a	3.11a	85.1c
2MDPI-10	62.28c	7.23c	102.6a	200a	3.24a	103.2c
3MDPI-1 + 50% CF	70.66ab	7.90bc	119.5a	274a	3.84a	148.3b
3MDPI-6 + 50% CF	70.81ab	8.71bc	119.1a	279a	3.86a	169.1a
2MDPI-10 + 50% CF	62.28c	9.18abc	118.8a	260a	3.39a	71.3c

*Different letters in each column show significant differences based on Duncan multiple range test at $P \le 0.05$.

Table 2. The impact of biofertilizers, Fe and Zn foliar application on yellow mustard performance.

Treatments	Plant height (cm)	Dry weight (g)	Number of branches	Number of pods	Grain yield ($g \cdot m^{-2}$)	1000 seeds weight (g)	Oil yield ($g \cdot m^{-2}$)
100% Chemical fertilizer	70.04bc*	235.51b	7.21d	114.3ab	220.15b	3.17a	116.7b
Nitrozist	74.52b	221.15c	9.32c	99.1bc	199.21c	3.07a	108.6b
Phosphozist	65.32c	234.21b	6.87d	91.2c	177.61c	3.11a	101.2b

Continued

Nitrozist + Phosphozist	75.15b	245.32a	12.21b	110.6ab	245.61ab	3.19a	149.5a
Fe foliar Ap.	66.66c	220.16c	9.84c	100.2bc	185.32c	3.01a	109.1b
Zn foliar Ap.	72.71b	218.21c	7.31d	92.7c	179.52c	3.14a	105.3b
Fe + Zn Foliar Ap.	80.13a	249.77a	14.05a	122.3a	273.09a	3.21a	153.2a

*Different letters in each column show significant differences based on Duncan multiple range test at $P \leq 0.05$.

3.2. Grain Yield and Yield Components

A comprehensive comparison table detailing various plant traits under distinct fertilizer treatments is provided for reference purposes. The study demonstrated the potential of cultivating yellow mustard for seed and oil production in a semi-arid research area. Yellow mustard plants successfully grew in all experimental plots, indicating the feasibility of dry land cultivation for this crop. Grain yield and yield components were not significantly impacted by different fertilizer treatments in the initial experiment, suggesting no notable differences between nitrogen-fixing bacteria isolates and chemical fertilizers. While the bacterial 3MDP-6 + 50% Chemical fertilizer treatment showed the highest grain yield, the differences among treatments were not statistically significant (Table 1) (Figure 1). The mean number of pods per plant was significantly lower in 3MDPI-6 treatment. 1000 seeds weight of plants were not affected significantly by different treatments in both experiments (Table 1 and Table 2).

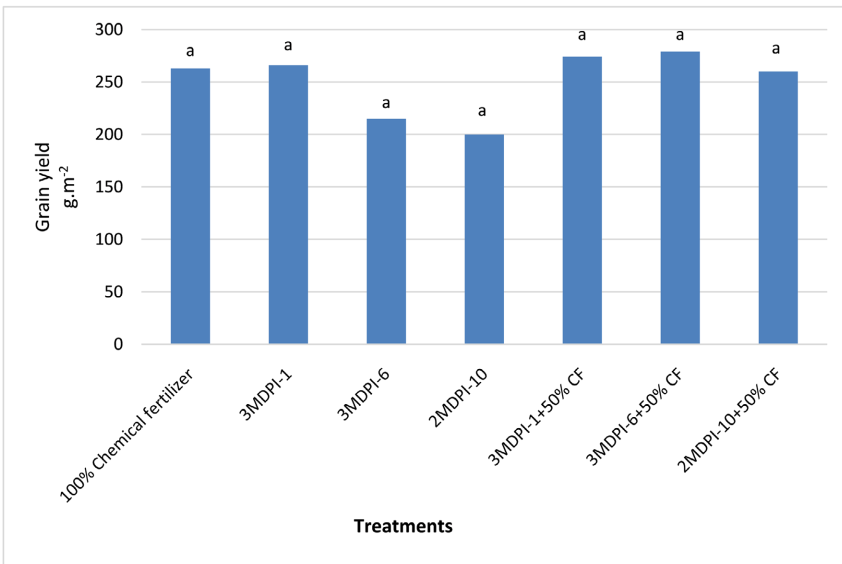

Figure 1. Effects of nitrogen fixing bacteria isolates and chemical fertilizers on yellow mustard grain yield in dry land conditions.

In contrast, the second experiment revealed significant effects of biofertilizers and foliar applications on yellow mustard grain yield and its components, except for 1000 seeds weight (Table 2). The mean number of pods per plant was observed in iron + zinc foliar application and plants which were treated by Phosphozist had the lowest mean number of pods. The iron + zinc foliar application resulted in the highest grain yield, while Phosphozist treatment had the lowest (Figure 2).

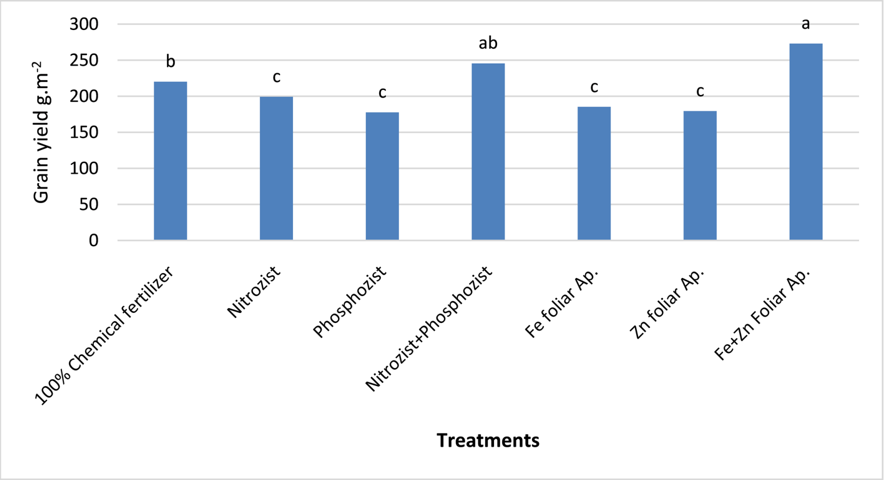

Figure 2. Effects of biofertilizers, Fe and Zn foliar application yellow mustard grain yield in dry land conditions. Different letters in each column show significant differences based on Duncan multiple range test at P ≤ 0.05.

3.3. Oil Yield

Oil production from mustard seeds was also significantly influenced by fertilizer treatments, with the highest oil yield seen in plants treated with 3MDPI-6 + 50% Chemical fertilizer in the first experiment and iron + zinc foliar application in the second experiment. Conversely, Phosphozist treatment yielded the lowest oil amount (Figure 3 and Figure 4).

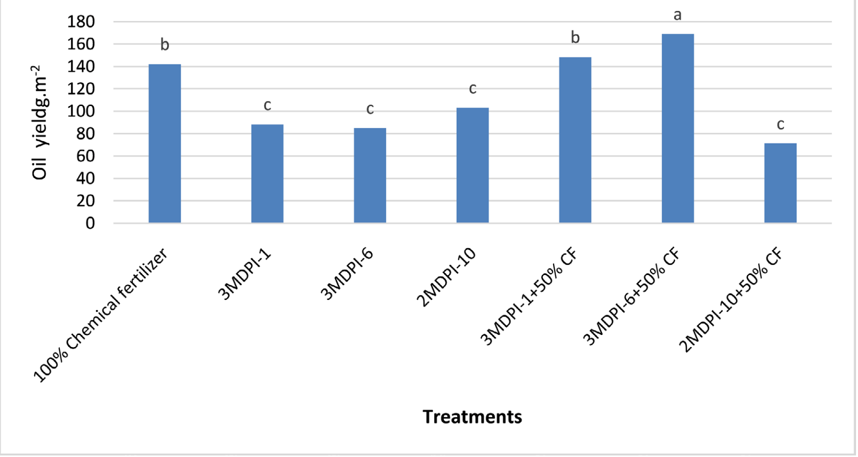

Figure 3. Effects of nitrogen fixing bacteria isolates and chemical fertilizers on yellow mustard oil yield in dry land conditions. Different letters in each column show significant differences based on Duncan multiple range test at P ≤ 0.05.

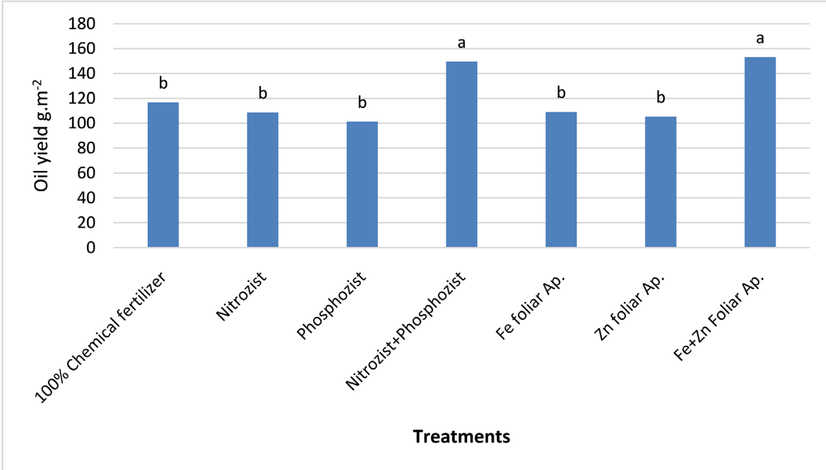

Figure 4. Effects of biofertilizers, Fe and Zn foliar application yellow mustard oil yield in dry land conditions. Different letters in each column show significant differences based on Duncan multiple range test at $P \le 0.05$.

4. Discussion

During the growing season, despite receiving only 68.2 mm of precipitation, all yellow mustard plots successfully matured and produced seeds.

In the initial trial, mustard plants treated with the bacterial isolate 3MDP-1 displayed superior height and lateral branch numbers. Conversely, in the subsequent experiment, plants treated with a combination of iron and zinc foliar application exhibited the greatest height, above-ground dry weight, and number of lateral branches.

The utilization of nitrogen-fixing bacteria along with a blend of biofertilizers and reduced chemical fertilizers (50%) yielded grain and oil outputs comparable to those achieved through 100% chemical fertilizer application. Furthermore, foliar application of iron and zinc significantly boosted grain and oil yields. Notably, treatments involving nitrogen-fixing bacteria, particularly isolates 3MDP-1 and 3MDP-6, outperformed the 100% chemical fertilizer treatment in terms of seed and oil production.

Integrated approaches incorporating nitrogen-fixing bacteria alongside a reduced amount of chemical fertilizer showed promise for transitioning towards sustainable cropping systems with lower reliance on chemical nitrogen fertilizers.

In contrast, the second experiment revealed that the highest grain yield resulted from utilizing a combination of iron and zinc foliar application while Phosphozist treatment led to lower yields. This experiment underscored the significance of micronutrient foliar applications like iron and zinc for enhancing seed and oil production in yellow mustard plants under dry land farming conditions. Additionally, it was noted that nitrogen biofertilizer proved more effective than phosphorus biofertilizer in this study.

The incorporation of biofertilizers in crops not only aids in biological nitrogen fixation but also facilitates phosphate solubilization in soil, thereby enhancing fertilizer efficiency. Studies have suggested a potential 25% reduction in chemical fertilizer usage by leveraging Azotobacter and Phosphorous-solubilizing bacteria in Indian mustard fields. Application of Azotobacter inoculants has been shown to enhance seed germination and initial plant vigor through growth-promoting substances [4] [11].

Apart from biofertilizers, essential nutrients like nitrogen, phosphorus, and potassium are critical for enhancing mustard quality. Nitrogen is pivotal for activating metabolic processes and energy transformation while phosphorus supports cell division, tissue growth, seed development, fruit formation, as well as flowering stimulation [12] [13].

Further research by Mondal *et al.* [5] indicated that applying biofertilizers can potentially reduce chemical fertilizer usage by 25% in mustard seed production without compromising seed yield levels. Our study findings suggest that in arid conditions, a 50% reduction in chemical fertilizer use is achievable by inoculating seeds with nitrogen-fixing bacteria or applying iron and zinc foliarly. This sustainable practice has the potential to lower production costs of mustard grain and oil while promoting environmental health. By integrating nitrogen-fixing bacteria and cutting down chemical fertilizers by half, it is possible to maintain mustard grain and oil yields, fostering more sustainable farming methods. The foliar application of iron and zinc plays a crucial role in enhancing crop yields under dry land conditions. This method can result in healthier products and reduced environmental impact.

The research indicates significant environmental advantages by decreasing reliance on chemical fertilizers, aiding in averting soil degradation and water contamination associated with excessive chemical fertilizer use. Biofertilizers not only boost soil health through improved microbial activity but also reduce the carbon footprint linked to manufacturing and transporting chemical fertilizers. Economically, farmers stand to benefit greatly from reduced chemical fertilizer usage leading to decreased input expenses.

Furthermore, the enhanced nutrient uptake efficiency facilitated by biofertilizers and micronutrient foliar applications can bolster crop yields and quality, potentially elevating market value. This dual advantage of cost savings and increased productivity renders this approach economically appealing. From an agronomic perspective, our study underscores the importance of integrated nutrient management as seen in the synergistic effects observed from combining biofertilizers with reduced chemical inputs for optimized nutrient availability and uptake. The positive outcomes of micronutrient foliar applications on plant growth emphasize the necessity for targeted nutrient interventions, particularly in deficient soils.

5. Conclusions

While our results are promising, challenges such as variations in soil types, climate conditions, and crop varieties should be addressed. Future research should focus on extensive field trials across diverse agro-ecological regions to validate findings and develop tailored recommendations for specific areas. Moreover, further investigation into the mechanisms governing interactions between biofertilizers, micronutrients, grain quality and plant physiology is essential for refining application strategies.

Overall, our study highlights the potential of biofertilizers and micronutrient foliar applications to sustainably enhance yellow mustard production in semi-arid regions as a viable alternative to heavy reliance on chemical fertilizers. Nitrogen-fixing bacteria could potentially be more efficient in decreasing the need for chemical nitrogen fertilizers. By incorporating these sustainable methods, farmers can attain cost-efficient and eco-friendly crop cultivation, contributing to the broader objectives of sustainable agriculture and food security.

Author Contributions

Zehtab Salmasi, S.: research idea development, supervising, writing-original draft and corresponding author. Nasiri, H. and Heshmati, R.: collecting data, and software. Sarikhani, M.R.: research idea, providing nitrogen fixing bacteria, writing-review and editing. Raei, Y.: advising, and writing-review and editing.

Conflicts of Interest

The authors report no conflicts of interest in this work.

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