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Integrated use of chemical and liquid biofertilizers for improved seed and oil yields in rapeseed (*Brassica napus*) in north-western Himalayas

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ABSTRACT

A present study was carried out during winter (*rabi)* season 2021–22 and 2022–23 at Shivalik Agricultural Research and Extension Centre, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishwavidalaya, Kangra, Himachal Pradesh to investigate the influence of fertility levels along with seed inoculations with liquid biofertilizers on seed yield, quality parameter and soil microbial population after harvest of rapeseed (*Brassica napus* L.) variety 'GSC-7'. The research was carried out using split plot design (SPD) with three fertility levels, viz. Control; 75% of recommended dose of fertilizer (RDF) and 100% RDF (120-60-40 kg NPK/ha) in main-plots in addition to six treatments of liquid biofertilizers, viz. *Azotobacter*; phosphate solubilizing microorganisms (PSMO); potassium mobilizing biofertilizer (KMB); NPK consortia + zinc solubilizing biofertilizer (ZSB); ZSB and control (no biofertilizer) as sub-plots, replicated thrice. The pooled data analysis of two-year recorded significantly higher values on number of primary and secondary branches/plant, siliquae/plant, seeds/siliqua, seed yield (1.89 t/ha), oil content (41.7%), oil yield of 792.2 kg/ha and soil microbial population in 100% RDF treatment followed by 75% RDF and control. The findings demonstrated that seed inoculation with *Azotobacter* and PSMO were found to be the most efficient biofertilizers. *Azotobacter* recorded significantly higher yield attributing characteristics and seed yield (1.68 t/ha), oil content (41.5%) and oil yield (718.1 kg/ha) along with higher soil microbial population after harvest of crop.

Keywords: *Azotobacter*, Biofertilizers, Seed yield, Oil content, Rapeseed, Soil microbial population

Over the past three decades, the oilseed sector has been a foremost rapidly growing areas of world agriculture, expanding at an annual growth of 4.1%, outpacing the growth of both agriculture and livestock products (Anonymous 2023). Gobhi sarson (*Brassica napus* L.) is an important oilseed crop after soybean in the country. Application of chemical fertilizers poses several dangers to human and animal health (Daniel *et al.* 2022). Biofertilizers contain living microbial inoculants which improve plant nutrition and nutrient uptake from the soil (Pradhan *et al.* 2022). *Azotobacter* transform elemental nitrogen into NH_3 form and also produces plant growth-promoting substances such as auxins, gibberellic acid and cytokinins that have significant effects on plant growth (Sumbul *et al.* 2020). PSB release organic acids, protons and siderophores and releasing soluble phosphate (Munda *et al.* 2018, Seenivasagan and Babalola 2021). Organic acid produced by potassium solubilizers can provide K^+ ions from K-bearing minerals by chelating which may dissociate the lattice structure and release K^+ into soil solution (Olaniyan *et al.* 2022). Zinc solubilizing biofertilizer (ZSB) synthesizes gluconic acid and ketogluconic acid that chelate Zn through carboxyl and hydroxyl groups to enhance Zn avaibility and uptake by plants (Nitu *et al.* 2020). The NPK consortia, comprising *Rhizobium*, *Azotobacter*, *Acetobacter*, phosphobacteria (*Pseudomonas*) and potassium-solubilizing *Bacillus* bacteria are crucial for boosting nitrogen fixation, phosphorus solubilization and potassium mobilization, thereby improving nutrient availability for plants and supports their growth (Gohil *et al.* 2021). Cultivation of oilseed crops are mainly undertaken in rainfed areas with poor input resources, which causes adverse effects on plant health, nutrition and ultimately crop production and yield. Because of the high cost along with negative impacts of synthetic fertilizers on soil fertility, plant and human health, it is not feasible to depend solely on conventional fertilizers. The combined application of biofertilizers with chemical fertilizers decreases dependence on chemical fertilizers, providing an environmentally friendly and cost-efficient

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approach that enhances long-term soil fertility and promotes sustainability in agricultural production. (Dass *et al.* 2008, Reddy *et al.* 2016, Seenivasagan and Babalola 2021). Considering above facts, the study on response of rapeseed to biofertilizers at varying fertility levels on yield, quality parameters and soil microbial population under north-west Himalayan conditions was undertaken.

MATERIALS AND METHODS

A present study was carried out during winter (*rabi)* season 2021–22 and 2022–23 at Shivalik Agricultural Research and Extension Centre, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishwavidalaya, Kangra (32° 09′ N latitude, 76° 22′ E longitude and 700 m amsl), Himachal Pradesh. The meteorological details for both seasons about various weather parameters were collected from Shivalik Agricultural Research and Extension Centre, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishwavidalaya, Kangra, Himachal Pradesh (Fig. 1). The crop growth period ranging between 41st to 15th standard meteorological weeks (SMW) during *rabi* season 2021–22 and 2022–23. The cumulative rainfall during an experiment were 286.8 and 221.8 mm in *rabi,* 2021–22 and 2022–23, respectively. The maximum temperature of 36.28°C was recorded in the 15th SMW (April) and minimum of 0.8°C was in the 4th SMW (January) whereas, the mean weekly relative humidity varied from 51–78% during *rabi* 2021–22, whereas, the maximum temperature of 33.3°C was recorded in the 15th SMW, while the minimum of about 4.2°C in the 2nd and 3rd SMW (January) and average weekly relative humidity varied from 70–89% during *rabi* 2022–23. The texture of field was silty clay loam having acidic *p*H of 5.65. Soil sample taken prior to the experiment from a depth of 0–15 cm was low in available nitrogen (278.3 kg/ha), medium in available phosphorus (17.6 kg/ha) and available potassium (225.8 kg/ha). The experiment was carried out using a split-plot design, allocating fertility levels in main plots, viz. control (no fertilizer); 75% recommended dose of fertilizer (RDF) and 100% RDF and six treatments of liquid biofertilizers, viz. *Azotobacter*; phosphate solubilizing microorganisms (PSMO); potassium mobilizing biofertilizer (KMB); zinc solubilizing biofertilizers (ZSB); NPK consortia + ZSB and control (no biofertilizer) in sub plots. The experiment was replicated thrice. Seed inoculation with liquid biofertilizers was done by soaking the seeds for 30 min in liquid biofertilizers and then dried in the shade for half an hour before sowing in field plots (gross plot size 11.76 m^2). The nitrogen was supplied by IFFCO fertilizer mixture (12:32:16) and urea, whereas the source of potash was the muriate of potash (MOP). As per main-plot treatments, entire dose of P, K and one-third dose of N was given as basal application. The remaining doses of N were given by urea top dressing at the vegetative and flowering stages. The recommended fertilizer dose was 120 kg N, 60 kg P_2O_5 and 40 kg K_2O/ha. The rapeseed variety '(GSC-7)' was sown manually by kera method with a 30 cm × 10–15 cm with a seed rate of 6 kg/ha. Weeding was done manually to manage the weeds in the experimental crop. The crop was manually harvested at maturity and the harvested produce of the net plot was kept for sun-drying followed by threshing. To evaluate the response of *gobhi sarson* to various treatments, observations were recorded on the yield attributing characteristics and seed yield. The siliquae were dried, threshed and cleaned, and then the seeds per siliqua was counted. 1000-seeds were selected from each sample and their weight was recorded. Soxhlet's extraction method was used to measure the oil content in seeds (A.O.A.C. 1960). Multiplying the oil content with respective seed yield resulted in oil yield.

$$\text{Oil (\%)} = \frac{\text{Oil weight (g)}}{\text{Sample weight (g)}} \times 100$$

$$\text{Oil yield (kg/ha)} = \frac{\text{Seed yield (kg/ha)} \times \text{Seed oil content (\%)}}{100}$$

Rain water use efficiency (RWUE) quantifies the yield obtained per mm of effective rainfall during the crop study period (Sharma *et al.* 2013). It was calculated for each treatment using the following formula:

$$\text{RWUE (kg/ha/mm)} = \frac{\text{Seed yield (kg/ha)}}{\text{Total effective rainfall (mm) during experiment}}$$

The effective rainfall was calculated using following FAO formula:

$$P_e = 0.8P - 25 \text{ (P>75 mm/month)}$$
$$P_e = 0.6P - 10 \text{ (P<75 mm/month)}$$

Where P and P_e are rainfall and effective rainfall (mm/month), (P_e is always ≥ 0; $\neq$ negative)

The statistical analysis for split-plot design was conducted using ANOVA (Analysis of variance) techniques explained by Cocharan and Cox (1957). Critical difference (CD) values among treatments were worked out at a 5% probability level, wherever F values were found to be significant. Data analysis was undertaken in OPSTAT software. When the data were similar during both the years, a pooled analysis was carried out and presented.

RESULTS AND DISCUSSION

Yield attributing characters: Application of recommended fertilizer dose (100% RDF) recorded significant increase in yield-related characteristics of rapeseed, including number of primary branches/plant (4.7), secondary branches/plant (3.1), siliquae/plant (236.3), seeds/siliqua (23.1) and 1000-seed weight (3.78 g) (Table 1). These results were notably superior to both 75% RDF and control (no fertilizer). The provision of sufficient chemical fertilizers effectively addressed the crop's nutritional demands, consequently augmenting the yield attributing characteristics. The application of 100% RDF ensured the provision of optimal levels of critical nutrients like NPK, pivotal for diverse physiological processes encompassing growth, development and reproductive phases in the plant, facilitated superior plant growth dynamics of traits contributing to yield (Tomar *et al.* 2018).

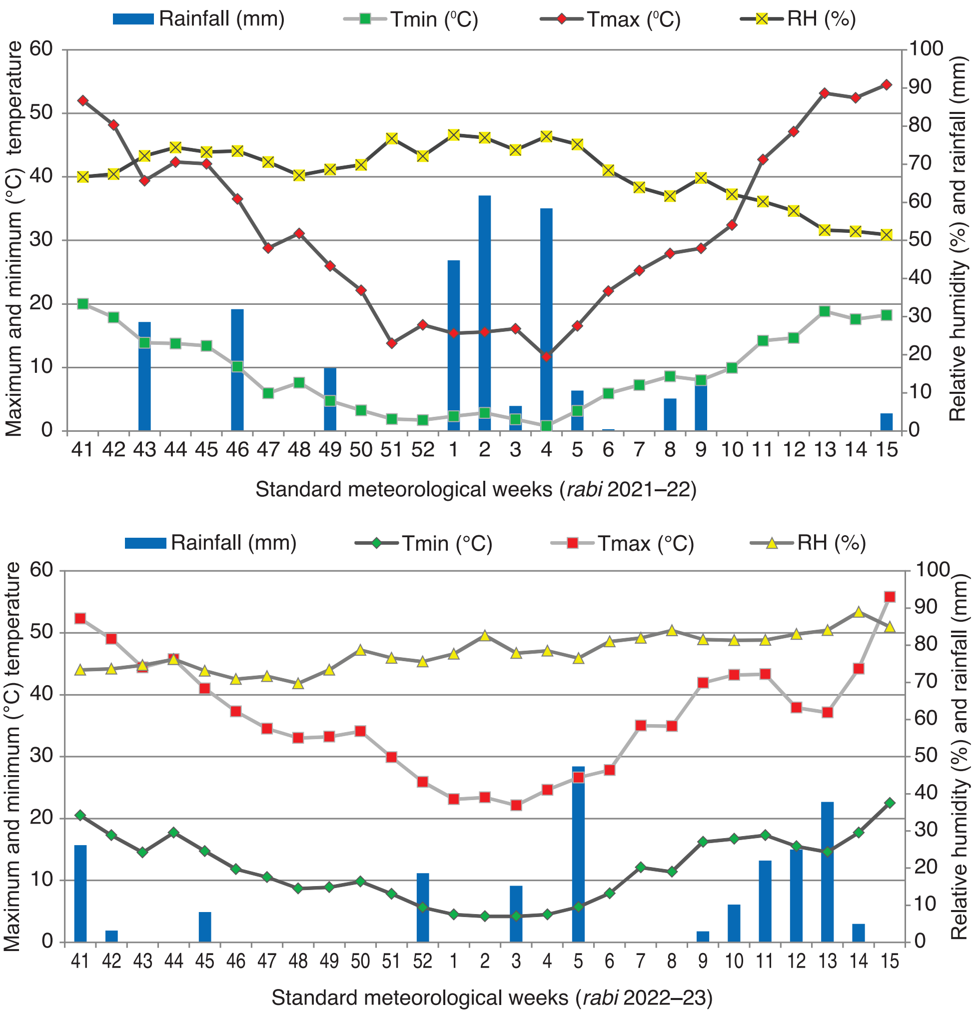

Fig. 1 Weekly weather data during *gobhi sarson* field experiment (*rabi* 2021–22 and 2022–23).

Among different biofertilizer treatments, seed inoculation with *Azotobacter* exhibited most pronounced effects, resulting in highest values for number of primary branches/plant (4.7), secondary branches/plant (2.8), siliquae/plant (209) and seeds/siliqua (23.3) statistically at par with PSMO and NPK consortia + ZSB for primary branches/plant and with PSMO for secondary branches/plant, siliquae/plant and seeds/siliqua. On mean basis, significantly higher value of test weight by 3.76 g was recorded with *Azotobacter* treatment followed by PSMO, NPK consortia + ZSB, ZSB and KMB over no inoculation (control) treatment. *Azotobacter* and other biofertilizers can produce growth-promoting substances, such as phytohormones and enzymes. These substances stimulate several physiological processes in plants namely cell division, elongation and differentiation, which contribute to increase yield attributing characters. The favourable effect of inoculation is due to increase in available nutrients and synergistic effect of co-inoculation on plant growth resulted in more yield attributes had been also observed by Hadiyal *et al.* (2017).

Seed yield: Significantly higher seed yield by 9.7% (1.92 t/ha) and 6.0% (1.86 t/ha) was recorded with 100% RDF over 75% RDF during 2021–22 and 2022–23, respectively (Table 2). This observation could be explained by the strong association between seed yield and the plant's yield-traits. Adequate nutrient availability through 100% RDF can enhance the efficiency of photosynthesis, helping plants more effectively transform light energy into chemical energy (sugars). This increased photosynthetic efficiency can lead to higher biomass production and allocation of resources towards yield formation (Dass and Bhattacharyya 2017).

Seed inoculation with liquid biofertilizers significantly increased the seed yield by 4.0–34.9% and 8.0–32.4% over control (no inoculation) treatment during 2021–22 and 2022–23, respectively. Significantly higher seed yield of 1.68 and 1.68 t/ha was recorded with *Azotobacter* during both cropping season that was 34.9 and 32.4% higher as compared to control (no biofertilizer) followed by PSMO treatment. On mean basis, 33.8, 25.8, 20.8, 16.6 and 6.1% higher seed yield was recorded by *Azotobacter*, PSMO, NPK consortia + ZSB, ZSB and KMB over uninoculated treatment. Beenish *et al.* (2018) observed a positive effect of biofertilizers on mustard seed yield. Phosphate solubilizing bacteria (PSB) are crucial for cell division, root elongation, production of phytohormones and also the essential components for ATP and ADP, which led to enhance yield attributing characteristics and the overall grain yield (Masrahi *et al.* 2023). Zinc solubilizing microbes increase the uptake of micro and macronutrients like N, P, K and Fe vis-a-vis also control the plant pathogenic microorganisms resulting in higher productivity and yield (Nitu *et al.* 2020). Overall, the inoculation of seeds with biofertilizers like *Azotobacter* enhances nutrient availability, promotes root growth, stimulates plant growth-promoting substances and provides protection against pathogens, all of which contributed to the increase in yield-attributing characteristics and ultimately, economic yield.

Quality parameters: Data on quality parameters revealed that an increase in inorganic fertilizers significantly increased the oil content and yield. On mean basis, significantly higher oil content (41.7%) and yield (792.2 kg/ha) were recorded with 100% RDF over 75% RDF and control.

Table 1 Effect of fertility levels and microbial consortia on yield attributes of rapeseed (Pooled data of 2 years)

Treatment	Primary branches/plant	Secondary branches/plant	Siliquae/ plant	Seeds/ siliqua	Test weight (g)
Fertility levels					
Control	3.6	1.1	130.1	20.8	3.42
75% RDF	4.3	2.6	216.6	22.2	3.64
100% RDF	4.7	3.1	236.3	23.1	3.78
SEm±	0.1	0.06	2.4	0.1	0.02
CD (P=0.05)	0.2	0.2	9.9	0.4	0.07
Microbial consortia					
Azotobacter	4.7	2.8	209.0	23.3	3.76
PSMO	4.5	2.7	204.9	22.8	3.68
KMB	4.0	1.9	187.6	21.3	3.54
ZSB	4.1	2.2	189.8	21.7	3.58
NPK consortia + ZSB	4.3	2.1	197.7	22.3	3.64
Control	3.5	1.9	176.9	20.6	3.47
SEm±	0.1	0.1	2.4	0.2	0.02
CD (P=0.05)	0.2	0.2	6.9	0.6	0.05

RDF, Recommended dose of fertilizer; PSMO, Phosphate solubilizing microorganisms; KMB, Potassium mobilizing biofertilizer; ZSB, Zinc solubilizing biofertilizer.

The addition of NPK fertilizers might have activated the enzymes responsible for producing oil, resulted in higher oil content (Singh and Pal 2011). Since the 100% RDF recorded higher seed yield and oil content, the same was reflected in the oil yield.

Biofertilizers favoured quality parameters positively as compared to uninoculated treatment. Seed inoculation with *Azotobacter* recorded 42.4% of oil content being statistically at par with PSMO (41.9%) followed by NPK consortia + ZSB, ZSB and KMB treatment over control (no inoculation). However, significantly higher oil yield was recorded with *Azotobacter* (718.1 kg/ha) followed by PSMO, NPK consortia + ZSB, ZSB and KMB by 46.80, 36.52, 29.86, 23.70 and 9.44% over control (489.2 kg/ha). Biofertilizers promote growth-promoting substances like enzymes and hormones, which regulate metabolic pathways associated with oil accumulation, including fatty acid synthesis, triacylglycerol formation and lipid storage processes, leading to elevated oil content in the seeds. Singh and Dutta (2006) and Kiani *et al.* (2013) also been reported that biofertilizers containing *Azotobacter chroococcum* fix atmospheric nitrogen and produce growth-promoting substances, which enhance nutrient uptake and activate lipid biosynthesis enzymes that directly improve metabolic efficiency, leading to increased the quality parameters and seed yield by 20–30% in Indian mustard.

Rain water use efficiency: Rainwater use efficiency is inversely proportional to cumulative seasonal rainfall from seed sowing to harvest. Consequently, due to higher seasonal rainfall, lower value was recorded in 2021–22 as compared to 2022–23 across all treatments. Rainfall is a key factor to determine the potential yield of rainfed crops and their nutrient uptake capacity, emphasizing its positive impact on yield in plots fertilized with 100% and 75% recommended fertilizer dose over no fertilizer treatment. Sharma *et al.* (2013) reported that a treatment receiving 100% RDF recorded a strong correlation between maize yield and seasonal rainfall. Rainwater use efficiency was higher in subplot treatments where seeds were inoculated with liquid biofertilizers. This might be attributed to sufficient soil moisture, which promotes nutrient mineralization and supports plant nutrient uptake.

Soil microbial population: Soil microbial populations are crucial biological factors that positively impact soil health and crop yield. Application of 100% RDF recorded significantly more fungal and actinomycetes population over 75% RDF and control whereas, higher bacterial population of 30×10^5 CFU/g soil with 100% recommended fertilizer dose remained statistically at par with 75% of RDF followed by control (Table 3). Adequate amount of nutrients (NPK) in soil promote the crop growth and better root activity stimulating secretion of the root exudates which promotes higher microbial growth in soil. Similar finding were also reported by Ratanoo (2020).

Pooled analysis of two-year data revealed that seed inoculation with biofertilizers showed significantly higher microbial population over control (no inoculation). On mean basis, significantly higher bacterial and fungal population were recorded with *Azotoabacter* inoculation followed by PSMO, NPK consortia + ZSB, ZSB and KMB over control (no inoculation). The maximum actinomycetes population was found in *Azotobacter* was statistically at par with PSMO treatment and followed by NPK consortia + ZSB, ZSB and KMB as compared to control in pooled

Table 2 Effect of fertility levels and microbial consortia on seed yield, quality parameters and rain water use efficiency of rapeseed

Treatment	Seed yield (t/ha)			Oil content (%)			Oil yield (kg/ha)			RWUE (kg/ha/mm)		
	2021–22	2022–23	Pooled	2021–22	2022–23	Pooled	2021–22	2022–23	Pooled	2021–22	2022–23	Pooled
Fertility levels												
Control	0.71	0.84	0.78	40.7	39.2	40.0	294.3	329.7	313.7	5.33	10.63	7.98
75% RDF	1.75	1.75	1.75	41.3	40.3	40.8	728.5	710.9	719.7	3.01	22.19	12.60
100% RDF	1.92	1.86	1.89	42.1	41.3	41.7	813.4	771.0	792.2	14.27	23.57	18.92
SEm±	0.01	0.24	0.01	0.2	0.2	0.1	4.9	12.6	5.3	0.09	0.30	0.19
CD (*P*=0.05)	0.05	0.99	0.05	0.7	1.0	0.4	19.9	50.7	21.4	0.37	1.23	0.80
Microbial consortia												
Azotobacter	1.68	1.68	1.68	43.3	41.5	42.4	732.7	703.5	718.1	12.50	21.31	16.90
PSMO	1.56	1.61	1.58	42.9	41.1	41.9	671.4	664.4	667.9	11.57	20.33	15.95
KMB	1.29	1.37	1.33	39.7	39.7	39.7	519.6	551.2	535.4	9.63	17.37	13.50
ZSB	1.48	1.45	1.47	41.8	39.9	40.9	626.4	586.8	605.1	11.00	18.38	14.69
NPK consortia + ZSB	1.51	1.52	1.52	42.5	40.5	41.5	649.6	621.0	635.3	11.26	19.29	15.27
Control	1.25	1.27	1.26	37.9	39.2	38.6	475.6	502.9	489.2	9.23	16.09	12.66
SEm±	0.02	0.01	0.01	0.2	0.3	0.2	13.1	9.6	7.6	0.20	0.23	0.21
CD (*P*=0.05)	0.08	0.05	0.04	0.5	0.9	0.5	38.0	27.8	22.1	0.60	0.70	0.65

RDF, Recommended dose of fertilizer; PSMO, Phosphate solubilizing microorganisms; KMB, Potassium mobilizing biofertilizer; ZSB, Zinc solubilizing biofertilizer; RWUE, Rain water use efficiency.

Table 3 Effect of fertility levels and microbial consortia on soil microbial population after harvest of rapeseed (Pooled data of 2 years)

Treatment	Bacteria ($\times 10^5$ CFU/g soil)	Fungi ($\times 10^3$ CFU/g soil)	Actinomycetes ($\times 10^3$ CFU/g soil)
Fertility levels			
Control	25.7	15.2	8.8
75% RDF	29.1	17.0	9.2
100% RDF	30.0	21.0	9.9
SEm±	0.3	0.2	0.1
CD (*P*=0.05)	1.3	0.8	0.4
Microbial consortia			
Azotobacter	31.3	19.9	9.9
PSMO	30.3	18.7	9.8
KMB	26.3	16.7	8.9
ZSB	27.0	17.3	9.2
NPK consortia + ZSB	29.5	17.9	9.3
Control	25.1	15.9	8.8
SEm±	0.3	0.2	0.1
CD (*P*=0.05)	0.8	0.7	0.2

RDF, Recommended dose of fertilizer; PSMO, Phosphate solubilizing microorganisms; KMB, Potassium mobilizing biofertilizer; ZSB, Zinc solubilizing biofertilizer; CFU, Colony–forming unit.

analysis. These findings align with the study by Emtsev *et al.* (2010), which demonstrated that biofertilizer inoculation positively influences the microbial community and function. Biofertilizers enhance the plant growth promoting rhizobacteria (PGPR) which are recognized for their antifungal and anti gram-negative bacterial properties. Moreover, this PGPR has been observed to be highly associated with and favored by canola roots (Lay *et al.* 2018).

The analysis of two–year data summarized that use of 100% recommended fertilizer dose significantly improved the yield attributes, viz. number of primary and secondary branches/plant, siliquae/plant, seeds/siliqua and 1000-seed weight that resulted in significantly more seed and oil yield, oil content, rain water use efficiency besides microbial populations in soil after crop harvest over other fertility levels. Seed inoculation with biofertilizers significantly enhanced the yield attributes, seed yield, oil content, oil yield and RWUE over uninoculated (control) treatment. *Azotobacter* and PSMO were found to be the most efficient biofertilizer that boosts crop yield (seed + oil) by improving soil microbial properties.

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