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SOIL SCIENCE

Field Efficacy of Bio-fertilizers and Bio-inputs to Improve Wheat (*Triticum aestivum*) Production under Alkaline Soil in Kachchh District of Gujarat

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ABSTRACT

With the aim of reducing chemical fertilizer application and improving the sustainability of wheat cultivation under alkaline soil, the present study investigated the effects of different biofertilizers and organic products on wheat productivity. The application of these products was applied in the soil through irrigation water as solitary or in consortia by three different ways of treatment, i.e. (1) single treatment; (2) double treatment (first and 21 days), and (3) triple treatment (first, 21 days and 45 days). We demonstrate that all the biofertilizers significantly enhanced wheat productivity as compared to the control treatment, but this was mostly observed with more than one treatment condition. The biofertilizers were applied in solitary and consortia treatments. Solitary treatment of *Azospirillium* spp, *Bacillus subtilis*, and *Pseudomonas fluorescence* was found effective. However, the Nitrogen consortium treatment of biofertilizers (*Azotobacter chroococcum* + *Azospirillium* spp) was more prominent as compared to solitary application. Our results suggest that more than one application of biofertilizers may be effectively exploited for sustainable wheat production in an eco-friendly way, but attention should be paid to the consortium approach or organic amendments during cultivation.

Highlights

- Bioinoculant showed a positive effect due to their PGP characteristics on wheat shoot growth
- Promising bacterial strains of Nitrogen fixers have potential as inoculation agents in eco-friendly wheat crop production under abiotic stress contributing to environmental sustainability.

Keywords: Alkaline soil, biofertilizers, organic farming, wheat production

Wheat (*Triticum aestivum* L.) is one of the largest growing cereal crops worldwide. This cereal crop plays a major role in fulfilling the increased demand for food to ensure food security. Fertilization is a main driving force in wheat cultivation to increase grain yield and quality (Zhang *et al.* 2014; Mon *et al.* 2016). For this, farmers applied a large dose of chemical fertilizers to improve the production of wheat. However, irregular use of chemical fertilizers and pesticides negatively impacts on the environment, including soil fertility, microbial diversity, water holding capacity, nutrient mobilization capacity, etc. (Xiao *et al.* 2019). In recent decades, agricultural scientists devoted their research towards organic farming and emphasized the use of organic manure, biofertilizers, biopesticides, and other organic amendments in cultivation practices to improve plant productivity (Abou-Aly *et al.* 2009; Rakshit *et al.* 2014; Cisse *et al.* 2019; Cortivo *et al.* 2020; Sarkar *et al.* 2020).

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A long term organic farming practices showed positive impacts on soil fertility, dry crop matter, grain yield, and nutritional value (Esmailpour *et al.* 2013). Application of organic fertilizers and biofertilizers help wheat plants to survive under abiotic and biotic stress conditions (Mahmoud *et al.* 2008; Dal Cortivo *et al.* 2020). Hence, the microbial intervention has great potential to improve plant productivity under adverse conditions also and reduce the dependency on chemical fertilizers (Kumar *et al.* 2017; Manjhi *et al.* 2016; Parihar and Rakshit 2016). In the presented study, we conducted different experiments with biofertilizers, and nutrient compounds to evaluate their effect on wheat productivity.

The Kachchh area of Gujarat, India is highly alkaline with more than 89% villages having pH more than 8, Organic content < 1, and deprived with nitrogen (N) content the main requirement for wheat growth (Un published report of Satvik, 2016). In addition, the alkaline nature would further reduce the availability of other nutrients like P, Fe, Zn for plants. Hence, the aims of the present study were designed to improve wheat productivity and nutritional quality growing in the soil of the Kachchh region through organic farming practices by applying different amendments including dung water, curd, rock phosphate, and different commercially available bio-fertilizers alone and in different combinations. To attempt this objective, experiments were designed with different nutrient solubilizing microbes i.e., a nitrogen fixer, phosphate solubilizer, potassium solubilizer, Arbuscular mycorrhize fungi, and bio-control fungi *Trichoderma*. The effect of these microbes on wheat productivity was also compared with other organic treatments such as dung water, curd water, and chemical fertilization.

MATERIALS AND METHODS

Study Area

Experiments were conducted in the Anjar region of Gujarat, India. The geographical location of the farm is 23.134143" N (latitude), 69.898209" E (longitude). The farm is a certified organic farm (certificate no. ORG/SC/1312/001634) with NPOP, EU, and NOP standards by IMO Control Pvt. Ltd (Fig. 1).

Treatments

- To get a sustainable source of nitrogen - mix plantation strategy with Ranjako (*Medicago sativa*) and leguminous crops (*Cicer arietinum*) as compared to alone and different combinations of N fixing bio-fertilizer like *Azotobacter chroococcum, Acetobacter diazotrophicus and Azospirillium* spp. were used.
- To get a sustainable source of phosphorous, application of rock phosphate alone as compared to the application of bio-fertilizer like *Pseudomonas fluorescens* and *Bacillus subtilis* as well as in the combination of rock phosphate and *Pseudomonas fluorescens* were used.
- Impact of application of Potash solubilizing bacteria (*Fraturia aurantia*) in high Potash (K) soil of Kachchh on wheat productivity were studied.
- Application of micronutrients (Fe 60%, Zn 20%, Mn 10%, Mg 10%) on wheat productivity were also studied.
- Application of fungus like *Trichoderma harzianum* (In addition to anti-fungal activity, the genus boosts germination rate, solubilizes various insoluble forms of phosphates, augments nitrogen fixation)
- *Arbuscular mycorrhizal* Fungi (Increases the uptake and mobilization nutrients) on wheat production were also studied.

The farm covered total of 20 Acres (80,000 m^2) of land. Out of which approximately 3,900 m^2 area was occupied for the present investigation and divided into 52 plots, each of 25 m^2 as shown in Fig. A. As per standard criteria, 0.5 m border area was eliminated from each plot for the calculation of production of the crop during harvesting to avoid cross-contamination of application of amendments by adjoining plots. Therefore, production occurred in 13.50 m^2 area was considered as the production of wheat in each plot. A total of twenty amendments was applied (including control). The details of the amendments, dose, and their application are given in table 1. The method of irrigation used was Check Basin (CB). The farmers were well trained for the uniform application of each amendment in each plot using CB method of irrigation. The seeds were sowed by Automatic Seed Drill along with a tractor. The date of Seed sowed was 22.11.2019, and the date of crop harvested was 30.03.2020.

The treatment was divided into three types, viz. T1

Table 1: Details of study design

Code No.	Amendments	No. of Applications/ Treatment	No. of plots	Dose (for 25 m^2 plot)
1	Soil	Control	Single	No Treatment
2	Wheat + Ranjko (*Medicago sativa)*	Mix Crop	Single	Supplementary (Leguminous) crop for Nitrogen fixation. 15 gram *Medicago sativa* seeds was sown along with wheat and 30 gram *Cicer arietinum* seeds were sown along with wheat
3	Wheat + Chickpea (*Cicer arietinum*)	Mix Crop	Single	
4	Micro Nutrient ($FeSO_4$ + $ZnSO_4$ + $MgSO_4$ + $MnSO_4$)	T1, T2, T3	Three	The standard dose for the application of micronutrient is 25 Kg/Acer, and therefore, 150 gm was taken for 25 m^2 plot from a bag containing (15 Kg of Fe, 5 Kg of Zn, 2.5 Kg of Mn and 2.5 Kg of Mg) and mixed in 1L water and applied in the study along with irrigation water.
5	Dung Water	T1, T2, T3	Three	The standard dose for the application of Gobar water is 400 Kg/Acer and therefore, 2.5 Kg of Gobar was mixed in 10L of water and used for 25 m^2 plot. The slurry was filtered and applied in the study, along with irrigation water.
6	Curd Water	T1, T2, T3	Three	The standard dose for the application of curd is 20 Kg/Acer, and therefore, 125 Gm of curd was mixed in 1 L water for 25 m^2 plot and applied in the study along with irrigation water.
7	*Azotobacter chroococcum*	T1, T2, T3	Three	The standard counts of microorganisms in 2 ml should be 10^{11} counts. Which should be dilute in 1 litre water and apply in one acer as recommended by Company. The calculation was done for 25 m^2 plot, based on 7 ml of suspension was taken and further mixed in 1L water and applied in 25 m^2 plot along with irrigation water.
8	*Acetobacter diazotrophicus*	T1, T2, T3	Three	
9	*Azospirillium* spp.	T1, T2, T3	Three	
10	*Pseudomonas Florescence*	T1, T2, T3	Three	
11	*Fraturia aurantia*	T1, T2, T3	Three	
12	*Bacillus subtilis*	T1, T2, T3	Three	
13	*Trichoderma harzianum*	T1, T2, T3	Three	
14	AMF (Arbuscular Mycorrhizal Fungi)	T1, T2, T3	Three plots	The standard counts of microorganism should be 100 gram × 3000 counts/Acer as recommended by Company. The calculation was done for 25 m^2 plot, based on 7 ml of suspension was taken, and further mixed in 1L water and applied in 25 m^2 plot along with irrigation water.
15	*Azotobacter chroococcum* + *Acetobacter diazotrophicus*	T1, T2, T3	Three	7 ml of each suspension was taken as described above and mixed in 1 L water, and applied in the study along with irrigation water.
16	*Azotobacter chroococcum* + *Azospirillium* spp	T1, T2, T3	Three	
17	*Acetobacter diazotrophicus* + *Azospirillium* spp	T1, T2, T3	Three	
18	*Azotobacter chroococcum* + *Acetobacter diazotrophicus* + *Azospirillium* spp.	T1, T2, T3	Three	
19	Rock Phosphate (RP)	T1	Single	The standard dose for the application of RP is 400 Kg/Acer therefore, 2.5 Kg of RP was taken for 25 m^2 plot. Due to the floating tendency of RP on water, the RP powder was mixed with soil in each plot before irrigation water was applied to avoid uneven distribution.

20	Rock Phosphate + *Pseudomonas F*	RP was given only a single time, and Pseudomonas F was given for T1, T2, T3	Three	2.5 Kg of RP was taken for 25 m^2 plot and applied as described above only. 7 ml of suspension was taken as described above in the present study.

refereed as a single treatment on day one along with irrigation water, T2 referred as two times treatments one was on day one along with irrigation water and second was on 21 days along with irrigation water and T3 refereed as three times treatment one was on day one along with irrigation water, second was on 21 days along with irrigation water and third was on 45 days along with irrigation water. Each plot has received an equal quantity of water and is irrigated on first, 21, and 45 days.

RESULTS AND DISCUSSION

The outcome of the study was summarized in table 2. The data were presented as Mean, and S.E. Significant analysis was performed as compared to control using paired student's t test. The result revealed that the application of micronutrients and dung water during T3 treatment was significantly effective at $p<0.5$ (4.05 ± 0.15 kg/5 sq. Mt. plot) as compared to control treatment.

The application of *Azotobacter chroococcum* and *Acetobacter diazotrophicus* in the solitary application was found to be non-effective during all treatments i.e., T1, T2, and T3. However, the application of *Azospirillium* spp. in T1 and T2 was found to be significantly effective at $p<0.5$ as compared to control.

The consortium treatment of *Azotobacter chroococcum & Acetobacter diazotrophicus*, and *Azotobacter chroococcum & Azospirillium* spp*, as well as Acetobacter diazotrophicus & Azospirillium* spp., was found to be significantly effective in T2 and T3 treatment as compared to control at $p<0.5$. The combination of all three Nitrogen-fixing bacterial species of biofertilizer, i.e., *Azotobacter chroococcum, Acetobacter diazotrophicus,* and *Azospirillium* spp. found to be significantly effective in T1, T2, and T3 as compared to control at $p<0.5$. Treatment of *Pseudomonas florescence* alone was found to be significantly effective during all treatments i.e., T1, T2, and T3. Treatment of *Pseudomonas florescence* along with Rock Phosphate was found to be significantly effective during treatment T2 and T3 as compared to control. Treatment of *Fraturia aurantia* was found to be significantly effective as compared to control at $p<0.5$ during T1 and T3 treatment. Treatment of *Bacillus subtilis* was found to be significantly effective at $p<0.5$ in all treatments i.e., T1, T2, and T3. Treatment of *Trichoderma harzianum* was found to be significantly effective as compared to control at $p<0.5$ during T2, while the application of *Arbuscular Mycorrhizal Fungi* was found to be significant during T3 treatment.

Organic amendments in farming practices are a sustainable approach for improving soil fertility, crop productivity in a cheaper and eco-friendly way. Biofertilizers including bacteria, fungi, cyanobacteria are reported to exert a beneficial impact on the nutritional value and yield of wheat plant (Karthikeyan *et al.* 2007; Meena *et al.* 2016; Meena *et al.* 2017; Cisse *et al.* 2019; Dal Cortivo *et al.* 2020). However, most of the studies are performed under laboratory or controlled condition; the positive effect of biofertilizers in open field may constrain due to dynamic environmental conditions. Microbes used in this study *Azotobacter chroococcum, Acetobacter diazotrophicus, Azospirillium* spp., *Pseudomonas florescence, Fraturia aurantia, Bacillus subtilis, Trichoderma harzianum,* and *Arbuscular mycorrhizal fungi* have already been successfully applied on different crops (Wu *et al.* 2005; Sivasakthivelan *et al.* 2013; Akladious and Abbas 2014; Wani *et al.* 2016; Subhashini *et al.* 2015; Parihar and Rakshit 2016; Parihar *et al.* 2019; Parihar *et al.* 2020a and 2020b; Murumkar *et al.* 2017; Prasad and Babu 2017). In the present study, these microbes were applied as single inoculants and a combination thereof through field application, with the aim to increase the yield of wheat. With these microbial inoculations, we expect nutrient solubilization process in the soil, i.e., potassium and phosphorus, and nitrogen-fixing ability, thereby increasing nutrient uptake efficiency of the plant and finally growth (Mohammadi and Sohrabi 2012; Vaishnav *et al.* 2013; Itelima *et al.* 2018). Indeed, more than one-time treatment of these microbial inoculations was found to increase

Table 2: Impact of the applications of different amendments on wheat production in organic farm (Production in Kg from 13.5 Sq. Mt. Plot)

Sl. No.	Treatment	T1	T2	T3
1	Soil - Control	3.88 ± 0.36	NA	NA
2	Wheat + Ranjko (*Medicago sativa)*	2.86 ± 0.34	NA	NA
3	Wheat + Chana (*Cicer arietinum*)	3.30 ± 0.51	NA	NA
4	Rock Phosphate (RP)	3.78 ± 0.38	NA	NA
5	Micro Nutrient	3.88 ± 0.53	3.85 ± 0.67	4.05 ± 0.15*
6	Dung water	3.72 ± 0.30	3.89 ± 0.66	4.00 ± 0.39*
7	Curd Water	3.62 ± 0.46	3.63 ± 0.23	3.98 ± 0.50*
8	*Azotobacter chroococcum*	3.84 ± 0.31	3.84 ± 0.68	3.69 ± 0.50
9	*Acetobacter diazotrophicus*	3.86 ± 0.65	3.70 ± 0.45	3.57 ± 0.67
10	*Azospirillium* spp.	4.97 ± 0.09*	3.98 ± 0.43*	3.80 ± 0.11
11	*Azotobacter chroococcum* + *Acetobacter diazotrophicus*	3.88 ± 0.55	3.93 ± 0.15*	4.06 ± 0.58*
12	*Azotobacter chroococcum* + *Azospirillium* spp	3.73 ± 0.33	4.20 ± 0.14*	4.30 ± 0.33*
13	*Acetobacter diazotrophicus* + *Azospirillium* spp	3.40 ± 0.52	4.03 ± 0.47*	4.18 ± 0.65*
14	*Azotobacter chroococcum* + *Acetobacter diazotrophicus* + *Azospirillium* spp	3.91 ± 0.14	4.17 ± 0.53*	4.22 ± 0.49*
15	*Pseudomonas florescence*	3.97 ± 0.52*	3.93 ± 0.48*	3.95 ± 0.33*
16	Rock Phosphate + *Pseudomonas*	3.87 ± 0.45	4.06 ± 0.34*	4.26 ± 0.85*
17	*Fraturia aurantia*	4.16 ± 0.17*	3.86 ± 0.58	4.15 ± 0.28*
18	*Bacillus subtilis*	4.07 ± 0.53*	4.48 ± 0.69*	4.01 ± 0.63*
19	*Trichoderma harzianum*	3.55 ± 0.10	4.08 ± 0.76*	3.73 ± 0.84
20	*Arbuscular mycorrhizal fungi*	3.53 ± 0.69	3.68 ± 0.14	4.15 ± 0.27*

** Significant at p<0.5 as compared to control using students t-test; Data are expressed as Mean ± SEM; NA- Not applicable; T1: Single treatment on day 1 along with water; T2: Two times treatment on day 1 and day 21 along with water; T3: Three times treatment on day 1, day 21, and day 45 along with water; 20 tonnes/acre Farm Yard Manure (FYM) application before wheat production.*

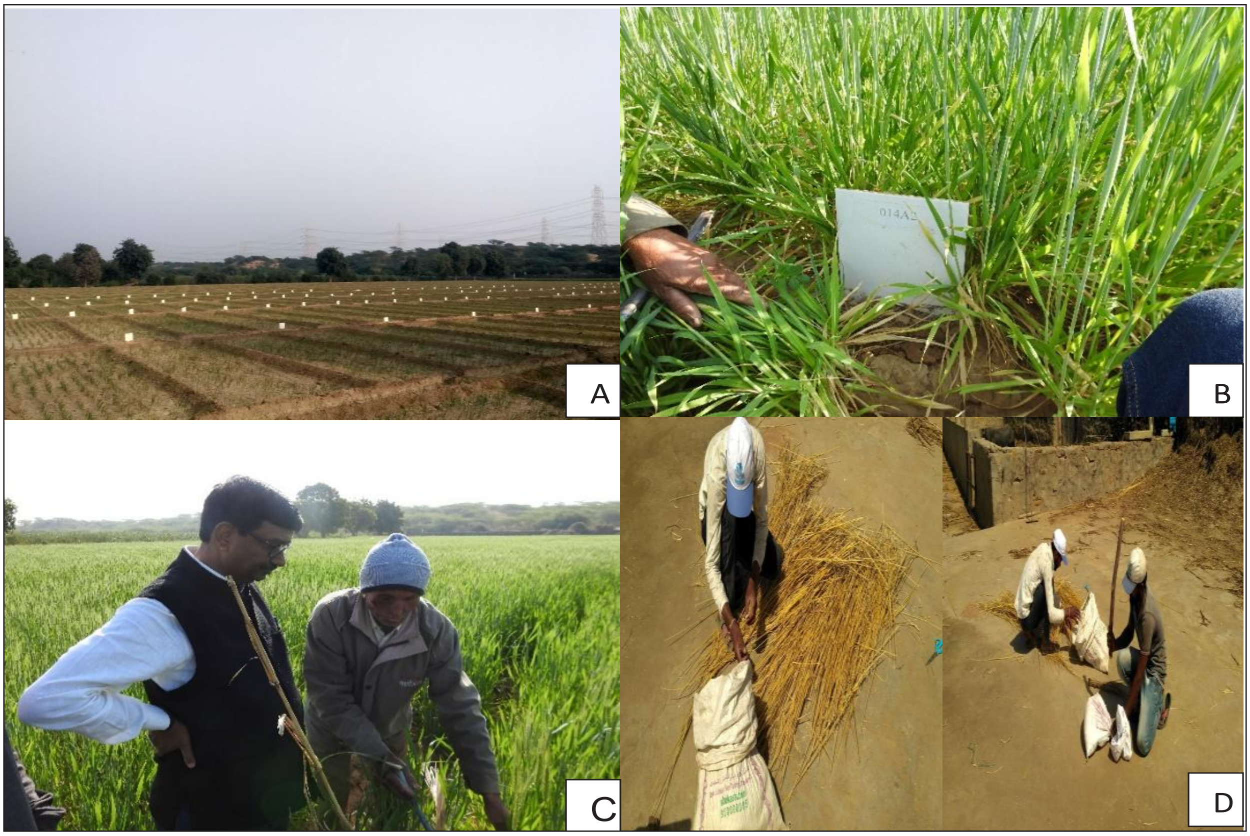

Fig. 1: (A) Plotting in the study area; **(B)** Systematic labeling of different plots; **(C)** Monitoring the study and discussion with farmers; **(D)** Harvesting of the crop on scheduled dates and collection of data

wheat productivity as compared to the control treatment. The highest productivity was observed with *Azospirillium* spp. inoculation alone. It might be due to its multiple actions in the soil, like mobilizing the other nutrients as well as maintaining pH of the soil (Fukami *et al.* 2018). In consortium treatment, the combined application of *Azotobacter chroococcum, Acetobacter diazotrophicus, Azospirillium* spp. was found prominent, and it might be due to the role of nitrogen-fixing bacteria in low nitrogen soil of semi-arid soil of Kachchh. Rock Phosphate + *Pseudomonas* treatment was also found to increase productivity as compared to individual treatment that suggests the solubilization of phosphorus by *P. fluorescence*. In addition, we found a clear positive effect of dung water and micronutrient application on wheat productivity as compared to control treatment.

CONCLUSION

This study has shown that bacteria, fungus, and AMF inoculation have a great impact on wheat plant growth under field conditions. More benefit was observed after three times the application of microbial inoculation. The benefits included the production of wheat gains (in Kg from 5.00 Sq. Mt. Plot). We confirmed the importance of Nitrogen-fixing microbial consortia (*Azotobacter chroococcum, Acetobacter diazotrophicus, and Azospirillium* spp.) in enhancing wheat growth and productivity. Despite moderate effects, the dung water and curd water tested here are also expected to increase productivity, which is of great importance in organic amendments.

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