



Efficacy of Commercial ZSB (Ami ZSB) on Growth and Yield of Wheat

Joshi Chinmay^{1*}, Zala Viren¹, Pandya Amit¹, Zala Harpal¹, Zala Vibhakshi¹, Zala Prakasha¹ and Trivedi Nidhi S²

¹Ami Agri Bioscience Pvt Ltd, Ahmedabad, Gujarat, India

²BioAgro Innovators LLP, Gandhinagar, Gujarat, India

*Corresponding Author: Joshi Chinmay, BioAgro Innovators LLP, Gandhinagar, Gujarat, India.

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Abstract

This study evaluates the effect of Zinc-Solubilizing Bacteria (Ami ZSB) on zinc availability and uptake in wheat (*Triticum aestivum* L.) to improve crop development, yield, and nutritional value. Zinc solubilization assays, bacterial isolation, and randomized complete block design field trials were all carried out in the lab and fields. The zinc-solubilizing potential of bacterial strains was tested on Bunt and Rovira medium with insoluble zinc sources such as zinc oxide and zinc carbonate. Also, Liquid broth experiments were used to investigate quantitative zinc solubilization. The results show that the Ami ZSB treatment considerably improved zinc solubilization, resulting in a higher plant biomass 93.71 g. Ami ZSB treated plants also had longer roots 17.3 cm, more tillers 9.3, total grains weight 13.57 g, and higher zinc concentrations in grains 40.28 ppm. These data indicate that Ami ZSB effectively increases zinc uptake, enhancing wheat growth and potentially providing a sustainable alternative to chemical fertilizers. This study emphasizes using biofertilizers like Ami ZSB to reduce dependency on chemical inputs, thereby promoting better and more sustainable agriculture methods.

Keywords: Zinc Solubilization Efficiency; *Triticum aestivum* L.; Biofertilization; Sustainable Agriculture; Grain Zinc Concentration; Biofertilizers; Zinc Enriched Fertilization

Abbreviations

ZSB: Zinc Solubilization Bacteria; SE: Solubilization Efficiency; Solubilization Index (SI); ZnO: Zinc Oxide; ZnCO₃: Zinc Carbonate; LB: Luria-Bertani; RCBD: Randomized Complete Block Design

Introduction

Wheat (*Triticum aestivum* L.) is a major staple crop globally, with annual harvests exceeding 700 million metric tons [1]. This crop helps supply billions of people's daily consumption of calories, providing food availability as the world's population expands [2]. Wheat constitutes more than 55% of the global consumption of carbohydrates and 20% of dietary calories [3]. Wheat's tolerance to various climates, along with its economic importance in world trade, highlights its essential role. As the world's population rises, there is an increasing need for more wheat output [4], demanding the development of alternative agricultural methods. Among the various micronutrients essential for wheat growth, zinc plays

a pivotal role in enzyme activation, protein synthesis, and overall plant health [5]. Zinc deficiency in wheat can cause stunted growth, lower grain formation, and poor crop quality, highlighting the need for productive ideas to manage nutrient shortages and maintain high yields [6].

In recent years, bio fertilizers have evolved as a sustainable alternative to chemical fertilizers, increasing soil nutrient availability through natural processes [7]. However, acceptance remains limited due to variable outcomes across agro-climatic environments and a lack of awareness of their mechanisms. While chemical fertilizers provide immediate availability of nutrients, their widespread use has resulted in environmental and economic issues such as soil erosion, greenhouse gas emissions, and water pollution, as well as increased costs for small-scale farmers [8]. Biofertilizers can address these difficulties and create healthier agricultural systems. Also, the availability of certain micronutrients, specifically zinc, is

frequently a limiting issue in agriculture, particularly in soils that are naturally poor or have been reduced due to intensive agriculture [9].

Micronutrients are required in small quantities, but they are important for plant growth and development [10]. Micronutrient deficiencies, particularly zinc, have a substantial effect on wheat productivity [11]. A lack of zinc includes chlorosis, reduced leaf size, and poor photosynthesis, all of which reduce overall plant health and output. Eliminating zinc deficiency is crucial for increasing wheat output and quality [12]. Fertilizers, including zinc chemical fertilizers such as zinc sulfate or zinc oxide, can remedy soil deficiencies and increase plant access to this element [13]. Furthermore, the use of zinc-enriched fertilizers, with improved soil treatment, aids in maintaining appropriate zinc levels and reducing subsequent deficiencies. These measures not only increase wheat growth and productivity but also improve the nutritional quality of the grain [14].

Zinc deficiency is common in soils worldwide, which leads to reduced growth, lower yields, and poor nutritional quality in wheat [15]. Zinc-solubilizing bacteria (ZSB) have shown potential for solving this problem by converting insoluble zinc components in the soil into forms that plants may easily absorb [16]. Biofertilizers, such as zinc-solubilizing bacteria (ZSB), are used to increase yield, nutritional quality, and environmental sustainability, improving agricultural productivity [17].

This study investigates the effect of zinc-solubilizing bacteria (Ami ZSB) on zinc absorption in wheat. The objectives of this study are to determine the effectiveness of (Ami ZSB) as a biofertilizer in increasing zinc availability and uptake in wheat crops, to assess its overall impact on plant growth and yield, and to investigate the broader implications of using (Ami ZSB) as a sustainable alternative to chemical fertilizers. In another experiment, zinc-solubilizing bacteria (ZSB) were applied to wheat, and the crop's zinc content, absorption, and nutrient efficiency improved significantly [18]. By addressing these goals, the investigation aims to focus on current gaps in our understanding of zinc solubilization in soils and its role in increasing crop productivity.

Materials and Methods

Qualitative estimation of (Ami ZSB)

Zinc solubilization assay

An incubation study was performed to assess the zinc-solubilizing capacity of isolated bacterial strains using different insoluble zinc sources: ZnO and ZnCO₃. The selected bacterial isolates were cultivated in Bunt and Rovira media enriched with an insoluble zinc source. Bacterial cultures were inoculated into the medium in triplicate to produce clear halo zones and incubated at 28 ± 1°C for seven days. The bacterial colony size and halo zones were measured using a measuring scale. Zinc Solubilization Efficiency (SE) was calculated as the ratio of the total diameter (including the halo zone and bacterial colony) to the colony diameter. The Solubilization Index (SI) was determined using the formula:

$SI = \frac{\text{halo zone diameter} - \text{colony diameter}}{\text{colony diameter}}$ Solubilization efficiency was calculated by using formula:

$SE = \text{Solubilization Efficiency}$

$SE = \frac{\text{halo zone diameter}}{\text{colony diameter}} \times 100$

Isolation and purification

The plate assay method was employed to measure the zinc-solubilizing ability of selected bacterial isolates [19]. Initially, the isolates were cultured in nutrient broth and incubated in a shaking incubator at 28 ± 1°C. Erlenmeyer flasks containing 50 mL of liquid Bunt and Rovira media, supplemented with 0.1% Zinc Oxide (ZnO) and Zinc Carbonate (ZnCO₃), were inoculated with 1 mL of the corresponding rhizobacterial culture. This method facilitated testing of zinc solubilization under controlled conditions. Uninoculated Bunt and Rovira media enriched with Zn compounds served as controls. The flasks were incubated in a shaking incubator at 28 ± 1°C for eight days. The pH level of the liquid broth was monitored at various intervals, and aliquots of the medium were centrifuged and purified. The amount of soluble zinc was assessed using an atomic absorption spectrophotometer, and the solubilized zinc content was calculated by subtracting the soluble zinc in the uninoculated control from that in the inoculated sample, expressed as grams of zinc per millilitre of culture.

Quantitative estimation of (Ami ZSB)

Zinc-solubilizing bacteria were isolated from rhizospheric soil using the serial dilution plate method, with Luria-Bertani (LB) agar

as the growth medium [20]. The LB medium consisted of tryptone (10 g/L), NaCl (10 g/L), yeast extract (5 g/L), and agar (20 g/L) [21]. To obtain pure cultures, the isolates were streaked on Bunt and Rovira media in succession. The medium included glucose (10 g/L), ammonium sulphate (1 g/L), potassium chloride (0.2 g/L), di-potassium hydrogen phosphate (0.1 g/L), magnesium sulphate (0.2 g/L), agar (15 g/L), and 0.1% zinc, with the pH adjusted to 7. Bacterial colonies exhibiting substantial growth and clear halo zones were selected, further purified, and preserved at -40°C in 20% glycerol.

Field experiment

Research site

The field experiment was conducted during the 2019–2020 growing season at the Ami Experimental Farm in Ahmedabad, Gujarat, using wheat (*Triticum aestivum L.*) as the test crop. Prior to sowing, the experimental field was meticulously prepared by ploughing and levelling.

Soil preparation

The experiment utilized a Randomized Complete Block Design (RCBD) with three variations. The treatments involved the application of zinc-solubilizing bacteria (Ami ZSB), with untreated plots serving as the control. Initial (Ami ZSB) dosages were applied before sowing wheat seeds using a seed drill. Various parameters, including plant biomass, root length, tiller number, total grain weight, and grain zinc concentration, were measured and evaluated.

Result and Discussion

Analysis of zone and colony diameters

The qualitative examination of zinc-solubilizing bacteria (Ami ZSB) showed a zone diameter of $17.8 \text{ mm} \pm 2.3 \text{ mm}$, representing the area where the bacteria exhibited zinc solubilization activity. Microbial colonies measured an average diameter of $1.2 \text{ mm} \pm 0.1 \text{ mm}$.

Plant biomass

The effect of zinc-solubilizing bacteria (Ami ZSB) on plant biomass was identified by comparing the control and Ami ZSB-treated plants. The control group had an average biomass of $71 \text{ g} (\pm 3 \text{ g})$, while the Ami ZSB-treated plants had a considerable increase, with

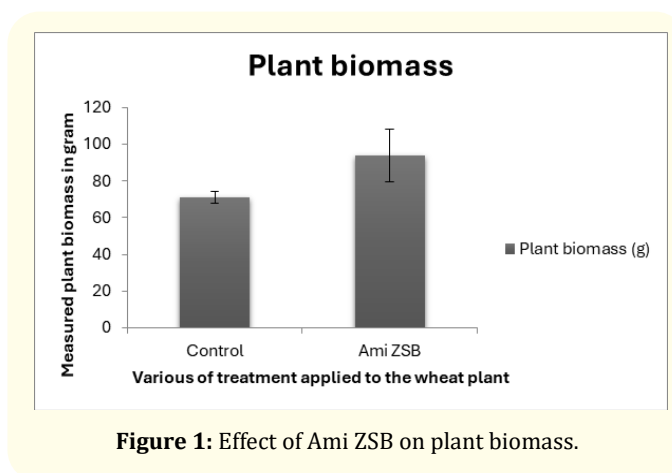


Figure 1: Effect of Ami ZSB on plant biomass.

an average biomass of $93.71 \text{ g} (\pm 14.2)$ as evaluated in figure 1. This significant growth augmentation in treated plants implies that Ami ZSB most likely enhances zinc availability, resulting in higher biomass accumulation. In another experiment, the zinc-solubilizing bacteria (ZSB) strain H 103 significantly increased plant biomass, yielding 92.83 g , substantially more than the 74 g produced by the control group [22].

Root length

The study of root length showed (figure 2) a notable improvement when treated with Ami ZSB in comparison to the control group. Ami ZSB treatment resulted in considerably longer root lengths $17.3 \pm 1.35 \text{ cm}$ compared to the control group $8.1 \pm 0.2 \text{ cm}$. In the related experiment on wheat, the addition of zinc-solubilizing rhizobacteria resulted in a considerable increase in plant height, reaching 15 cm , the highest recorded value among the treatments [23].

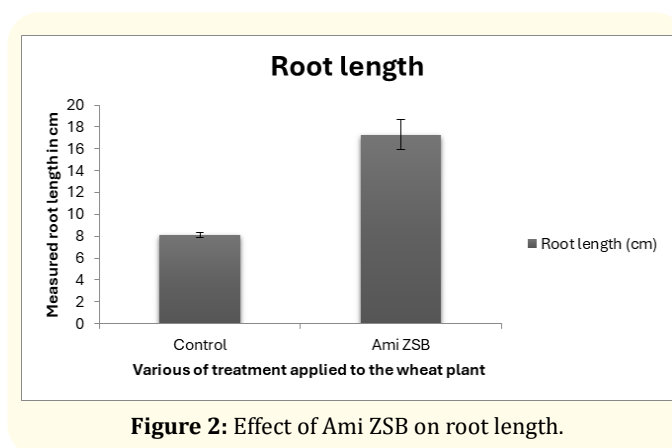


Figure 2: Effect of Ami ZSB on root length.

Number of tillers

This study in figure 3 observed clear differences in tiller output between the control and treatment groups. The control group had 2.1 ± 0.7 tillers, whereas Ami ZSB dramatically increased tiller numbers to 3.9 ± 0.34 . This growth highlights Ami potential of (Ami ZSB) as an effective biofertilizer, stimulating tiller formation in wheat and resulting in improved growth and yield outcomes. Similar trend of result were seen in other study on *Oryza sativa* found that using *Bacillus spp.* as a source of zinc-solubilizing bacteria (ZSB) resulted in an average of 9.1 ± 0.21 tillers per plant [24].

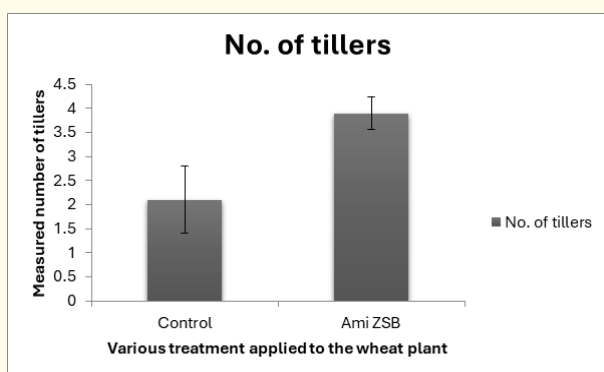


Figure 3: Effect of Ami ZSB on number of tillers.

Total grain weight

The application of zinc-solubilizing bacteria (Ami ZSB) had a notable effect on wheat grain weight, indicating the importance of micronutrient bioavailability in crop production. Figure 4 showed that the control group had no ZSB treatment and had a total grain weight of 7.13 ± 0.3 g, indicating the baseline yield under typical cultivation conditions without biofertilizers treatment. The treatment with Ami ZSB produced a higher grain weight, reaching 13.57 ± 0.21 g. This is a high difference, approximately doubling the grain weight compared to the untreated control. However, our findings on the wheat crop are consistent with previously reported wheat research studies, where the addition of Zinc-Solubilizing bacteria (ZSB) alone resulted in an 11% increase in 100-grain weight, totaling 38.81 g, as compared to the control. This improvement shows that ZSB improves grain development by increasing zinc availability, which results in greater grain weight [25].

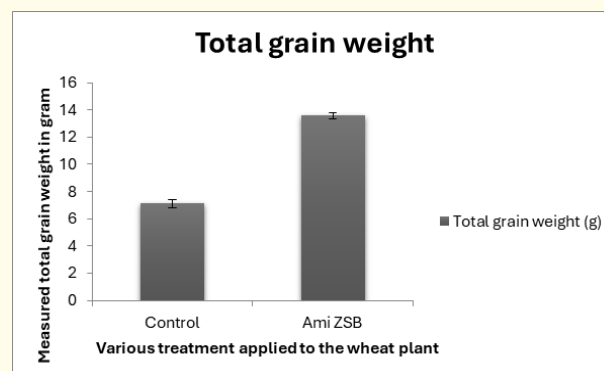


Figure 4: Effect of Ami ZSB on total grain weight.

Grain zinc concentration

The addition of ami zinc-solubilizing bacteria considerably increased the zinc (Zn) concentration in wheat grains as compared to the control. The Zn concentration in the untreated control group was 23.56 ppm (± 1.2), while grains from plants treated with Ami ZSB had a higher concentration of 40.28 ppm (± 3.5) depicted in figure 5. There is an enormous improvement after applying (Ami ZSB) to wheat crops. In a separate trial with ZSB on wheat, researchers observed a grain zinc concentration of 35 mg per kg, much greater than that of the control group, demonstrating a more resilient impact of ZSB on wheat [26].

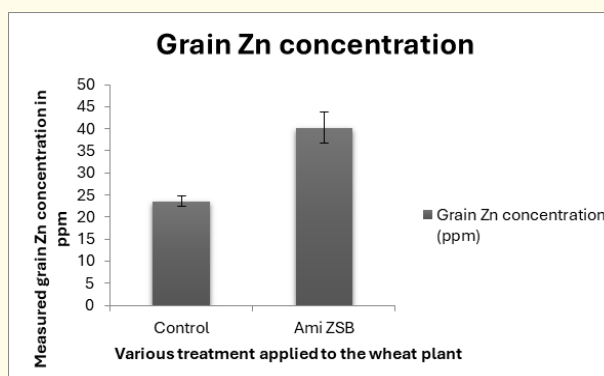


Figure 5: Effect of Ami ZSB on grain zinc concentration.

Conclusion

This result of the study show that zinc-solubilizing bacteria (Ami ZSB) can enhance zinc uptake in wheat (*Triticum aestivum L.*), resulting in considerable improvements in growth metrics such as plant biomass, root length, tillers number, and grain weight. Furthermore, the increase in grain zinc concentration indicates the potential of ZSB to alleviate zinc shortages in wheat production. These findings demonstrate position Ami ZSB as a sustainable bio-fertilizer and a viable alternative to chemical fertilizers, helping to improve crop output and nutritional quality. The study emphasizes the significance of using bio fertilization solutions to meet rising global wheat demand while lowering reliance on chemical inputs and encouraging ecologically responsible agricultural practices.

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