

The effects of bulk, biologic and nano-form fertilizers on *Zea mays* growth under irrigated circumstances

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Abstract. In practical plant biology, nanotechnology has involvements on every step of cropping, such as early growing, maintenance, harvesting and post harvesting and it has caused remarkable changes in findings solutions for facing problems. A trial was done to study the effects of different fertilizers on maize performance. The trial compared NPK bulk fertilizer, synthetic nano-sized fertilizers (boron, zinc, and complete), and biological fertilizers. Analyzing the data through principal component (PC) analysis indicated that the PC1 and PC2 explained for 56 and 27% of the variability in the dataset. Synthetic nano-zinc and nano-boron emerged as the most promising fertilizers, showcasing superior performance in terms of yield performance and yield components. A vector-tool biplot highlighted a robust positively correlation between chlorophyll content and straw yield, along with similar trends in grain yield and number of kernels per ear. Conventional bulk fertilizer (NPK) showed relatively lower efficiency across most evaluated traits. Based on ideal trait biplot, biological yield and stem diameter exhibited similar properties like to ideal trait, while oil percentage and hundred grain weight demonstrated unfavorable performance across treatments. This analysis underscores the efficacy of the treatment × trait biplot in elucidating relationships among traits and facilitating visual comparisons between different fertilizers. Overall, the findings underscore the significant enhancement of various maize cultivation traits through the application of synthetic nano-zinc and boron fertilizers, particularly in full irrigation condition.

Keywords: bulk fertilizer, nano-complete, nano-zinc, treatment by trait interaction

Introduction

Corn (*Zea mays* L.) is a versatile crop known for its adaptability to diverse production environments. It ranks as the third most significant crop globally, following wheat and rice, in terms of cultivation area, production output, and grain yield. However, in semi-arid regions, water scarcity and drought stress pose considerable challenges to crop productivity (Zou *et al.*, 2021). Food security in these regions relies heavily on the cultivation of plants that exhibit high tolerance to water deficits. Approximately 20-25 percent of maize-growing areas experience the harmful impacts of environmental stresses and the impact of water scarcity on maize grain yields varies across different stages of crop growth (Meng *et al.*, 2016). Nonetheless, selecting for stress tolerance remains challenging due to the complexity of genotype $\times$ environment interactions and insufficient understanding of tolerance mechanisms. Studies have shown that water scarcity negatively impacts phenological traits and root properties in maize. Grain yield and its components in maize are governed by a complex interplay of genes that respond to water scarcity with varying degrees of adaptability. Consequently, maize yield losses due to drought stress can range up to 76%, depending on the severity, timing, and stage of drought occurrence. Chukwudi *et al.* (2021) observed a decrease in grain yield of approximately 40% under drought stress, primarily attributable to reductions in kernel weight and kernel number.

Effective nutrient management stands out as a critical factor influencing maize performance, with responses to mineral fertilization varying depending on fertilizer type, application timing, and soil conditions. Moreover, the efficacy of fertilization is influenced by environmental factors such as climate and soil moisture content (Chen *et al.* 2018). While the conventional recommendation for maize typically involves the application of NPK (nitrogen, phosphorus, and potassium) fertilizers, their application under water-limited conditions remains inadequately explored. Biological fertilizers also play a pivotal role in crop production and soil fertility enhancement. These fertilizers, referred to as bio-fertilizers, because they contain living microorganisms that augment the providing of essential nutrients to host plants via nature-based ways such as fixing of nitrogen and solubilizing of phosphorus (Kumar *et al.*, 2018). However, the effects of biological fertilizers under water stress conditions are not yet fully understood. Micronutrients, required by plants in minute quantities, typically maintain concentrations below 100 parts per million (ppm). Among these micronutrients, zinc and boron are pivotal for various metabolic processes. Zinc, a constituent of numerous enzymes and proteins, plays crucial roles in growth hormone production and internode elongation (Rudani *et al.*, 2018). Boron, essential for both vegetative and reproductive growth, contributes to the integrity

of cell walls by binding to pectic polysaccharides. Additionally, it facilitates cell division, influences plasma membranes and phenol metabolism, and is indispensable for nitrogen fixation (Kohli *et al.*, 2023). However, boron can become toxic at levels slightly exceeding those necessary for normal growth.

In semi-arid regions, micronutrients tend to be readily adsorbed to soil particles, with their availability decreasing as soil pH rises, so nano-fertilizers offer a potential solution to this issue (Janmohammadi and Sabaghnia, 2023). These next-generation fertilizers contain nano-scaled active nutrients and controlled-release kinetics to target specific sites, effectively serving as smart issues. Nano fertilizers significantly enhance the efficiency of nutrient usage compared to conventional bulk fertilizers, primarily due to targeted delivery and slow or controlled release (Dimkpa *et al.*, 2020). The superiority of nano-based fertilizers lies in their novel and improved physical, chemical, and biological properties, driven by their high surface area-to-volume. While some information exists on the foliar application of certain nano-micronutrient fertilizers on specific crops, there remains a lack of sufficient data regarding the efficacy of soil-applied nano-fertilizers under conditions of limited irrigation. Hence, this study aims to assess the impacts of bio- and nano- based fertilizers on maize performance, its components, and some other morphological traits under limited irrigation conditions.

Materials and methods

Trial

The study took place at the research field of the Agricultural and Natural Resources College of Moghan, located in northwestern Iran (latitude 39° 41' N and longitude 47° 32' E). Situated in a Mediterranean-type climate zone, the region experiences rainfall predominantly from May to October. As per data from the ParsAbad, Moghan. Maximum temperatures reach 31.4 °C in August, while minimum temperatures drop to 1.4 °C in January. The average yearly precipitation stands at 389.5 mm. Planting was conducted manually, with two seeds per hole on flat ground, followed by thinning to achieve the desired population densities soon after emergence. To ensure uniform germination, emergence, and establishment, all trial plots received an initial irrigation. Each plot comprised seven rows, each 5 meters in length, with row spacing set at 65 cm and intra-row spacing at 20 cm, aligning with recommended commercial densities for the site. Surface normal irrigation, reaching up to 100% of field capacity, was administered on the vegetative step. Irrigation during this period was scheduled when half the depth of the root zone approached a 50% depletion level, with a net irrigation water depth of approximately 120 mm.

Treatments

The clay loamy soil in the area boasted sufficient depth, exceeding 1 meter, soil characteristics included a clay loam texture, pH of 7.5, electrical conductivity (EC) of 0.94 ds/m, and low nutrient content (0.03% nitrogen, 0.01% phosphorus, and 0.02% potassium). Treatments encompassed various options: a control group receiving no fertilizer (control), bio-fertilizers including nitrogen (Bio-N), and phosphorous (Bio-B). Additionally, nano-boron (Nano-B), nano-zinc (Nano-Zn), complete nano-fertilizer (Nano-C), and bulk NPK (180:100:50 kg ha⁻¹ urea, superphosphate, and sulphate of potash) fertilizer were included (Table 1). Nitrogen bio-fertilizer composed of the promoting rhizobacteria consortium (107 CFU/ml; including *Azotobacter chroococcum* + *Azospirillum lipoferum* species. Also, phosphorus bio-fertilizer included phosphate solubilizing rhizobacteria such as *Pseudomonas* and *Bacillus*. Half of the nitrogen and all of the phosphorus and potassium were performed pre-sowing, while the second half of nitrogen was top-dressed after a sowing. Bio-fertilizers were applied via seed inoculation just before planting. The nano-chelated complete fertilizer contained several essential elements at specified concentrations. Nano-fertilizers were administered three times via foliar spray at a 2000 ppm rate during the 9-leaf stage, stem elongation, and heading stages. All farming managements were uniformly applied to all plots throughout the experimental period. The synthesized nano-fertilizers were obtained from the SepehrParmis Co., Iran.

Table 1. The applied fertilizer treatments on maize

#	Type	Code	Name
1	---	Control	No- fertilizer application
2	Bio-fertilizer	Bio-N	Nitrogen bio-fertilizer
3		Bio-B	Phosphorous bio-fertilizer
4	Nano-fertilizer	Nano-B	Boron nano-fertilizer
5		Nano-Zn	Zinc nano-fertilizer
6		Nano-C	Complete nano-fertilizer
7	Chemical-fertilizer	NPK	Nitrogen, phosphorous, and potassium

Traits

The About ten randomly selected plant samples were chosen from each plot to determine leaf area (LA), kernels per ear (KE), ear diameter (ED), ear length (EL) and stem diameter (SD). At the harvesting stage, three 4-meter rows of maize were harvested from the center of each plot to determine grain yield (GY), biomass yield (BY), straw yield (SY), hundred grain weight (HGW) and harvest index (HI). Relative water content (RWC) was recorded at the beginning

of the grain development stage. Oil percentage (OP) was recorded via a near-infrared tool while relative water content (RWC) and chlorophyll (CHL) were recorded at the starting of the grain formation time.

Data analysis

The biplot model as the treatment by trait (TT) interaction was applied with GGEbiplot application (Yan, 2001) via blow equation.

$$\frac{\alpha_{ij} - \beta_j}{\sigma_j} = \sum_{n=1}^2 \lambda_n \xi_{in} \eta_{jn} + \varepsilon_{ij}$$

where α_{ij} is the fertilizer i for character j, β_j is the average of fertilizers in character j, σ_j is the standard deviation of character j, λ_n is the lambda for PC (principal component) n, ξ_{in} is score for fertilizer i on PCn, η_{jn} is score for character j on PCn, and ε_{ij} is the error term.

Results and discussion

The fitted TT biplot fourmula elucidated 83% of the variability in the data illustrating the performance of maize across various fertilizers. Notably, the first two PC models determined for 56% and 27% of this variability, respectively. This significant portion of variation underscores the intricate interplay among the measured traits across the different fertilizer treatments (Begam *et al.*, 2024). As emphasized by Janmohammadi *et al.* (2016), the key structure among the characteristics must be evident in the fitted model, and the success of the TT biplot model hinges on identifying the first two PC axes. However, in line with the report of Sabaghnia *et al.* (2016), and Porkabiri *et al.* (2019), it has been proposed that two PC axes are adequate for predictive modeling in analyses of two-way datasets such as the TT biplot model. Hence, the interaction between the seven fertilizers treatments and the thirteen traits in current investigation was most accurately predicted by the PC1 and PC2.

In the polygon-too (Fig. 1), the traits are regarded as the tester, while the treatments serve as the entries. This figure effectively showcases which treatment(s) excel in specific traits and in this biplot, the fertilizer(s) positioned at each vertex indicate the best or worst performers in terms of the characteristics found within the section. From Fig. 1, it's evident that the Nano-Zinc fertilizer (Nano-Zn) treatment outperformed others in all traits such as grain yield (GY), except straw yield (SY), chlorophyll content (CHL) and oil percentage (OP). This suggests that Nano-Zn can be deemed as the optimal fertilizer for maize production, particularly excelling in these traits. Suganya *et al.* (2020) corroborated that the usage of zinc can enhance maize performance and other characteristics.

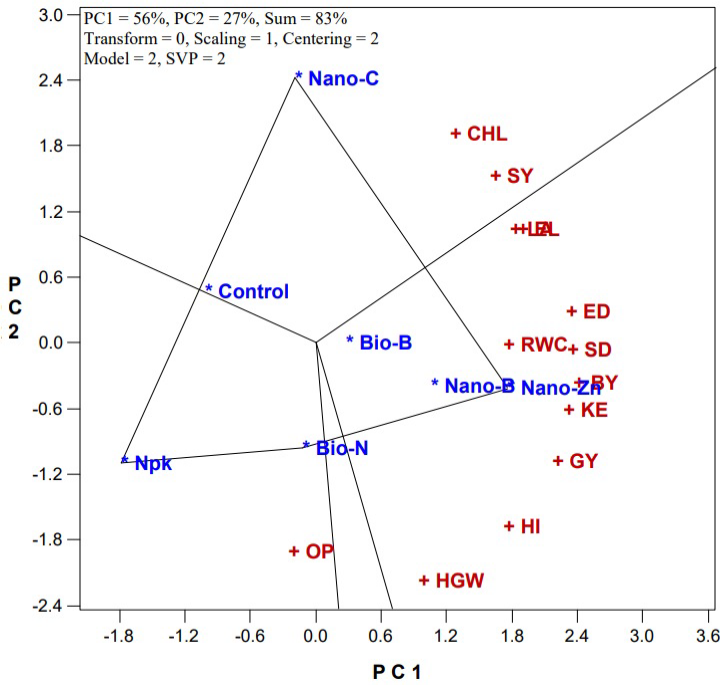


Figure 1. Which won where tool of treatment by trait (TT) biplot.

Traits are: leaf area (LA), kernels per ear (KE), chlorophyll content (CHL), ear diameter (ED), grain yield (GY), ear length (EL), stem diameter (SD), relative water content (RWC), biomass yield (BY), straw yield (SY), hundred grain weight (HGW), harvest index (HI) and oil percentage (OP).

Similarly, Ahmad *et al.* (2024) noted that maize grain yield increased with zinc fertilization, significantly boosting both nitrogen uptake and yield. The nano-complete fertilizer (Nano-C) emerged as the top-performing fertilizer for CHL and SY traits while conventional bulk NPK fertilizer (nitrogen + phosphorus + potassium) proved to be the best treatment for the oil percentage (OP) trait. Despite the Nano-Zn following to Nano-B demonstrating prowess in grain yield and yield component traits, they didn't excel in important traits like oil percentage and chlorophyll content. This suggests that yield properties may not necessarily correlate with oil percentage or chlorophyll content. Similarly, Farnia *et al.* (2015) found that nano-form fertilizers of zinc and boron improved yield components and performance in maize, aligning well with our findings.

In the vector-view TT biplot, vectors extend from the biplot origin approximate the association of traits by the angle cosine (Janmohammadi *et al.* 2017). The relative length of the vectors indicates the remarkable variability described by the model, with all measured traits contributing except for plant height (Fig. 2).

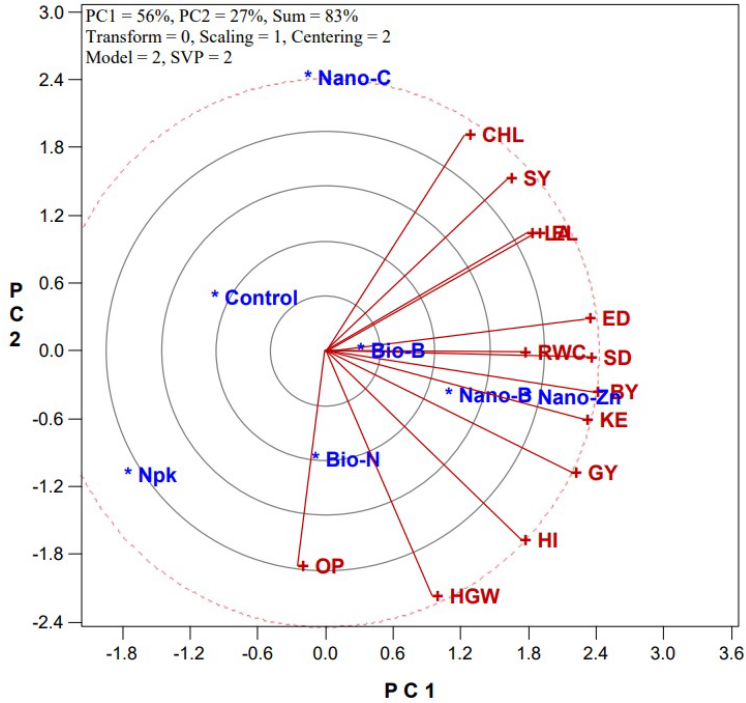


Figure 2. Vector tool of treatment by trait (TT).

Traits are: leaf area (LA), kernels per ear (KE), chlorophyll content (CHL), ear diameter (ED), grain yield (GY), ear length (EL), stem diameter (SD), relative water content (RWC), biomass yield (BY), straw yield (SY), hundred grain weight (HGW), harvest index (HI) and oil percentage (OP).

Notably, the relationships unveiled by this model include: (i) A positively association between CHL and SY, between ear length (EL) and leaf area (LA), between kernels per ear (KE) and biomass yield (BY), between grain yield (GY) and kernels per ear (KE), and between relative water content (RWC) with stem diameter (SD), evident from their angles. (ii) A relatively zero association between oil percentage (OP) with kernels per ear (KE) and biomass yield (BY), and between GY with SY and CHL, indicated by nearly perpendicular vectors. (iii) A negative association between chlorophyll content (CHL) and oil percentage (OP), illustrated by large obtuse angles (Fig. 2). While most of these predictions align with Pearson's correlation coefficients (Results are not shown), some discrepancies arise because the TT biplot method explains less than 100% of the total variation (in this study, 83%). Although, these conclusions contain minor errors, the TT biplot offers predictions on the overall dataset pattern, making them likely more reliable than individual observations (Yari *et al.*, 2018). Significant positive

correlations between yield performance and kernels per ear as well as significant positively correlation between ear length and leaf area have been reported (Zhang *et al.*, 2020).

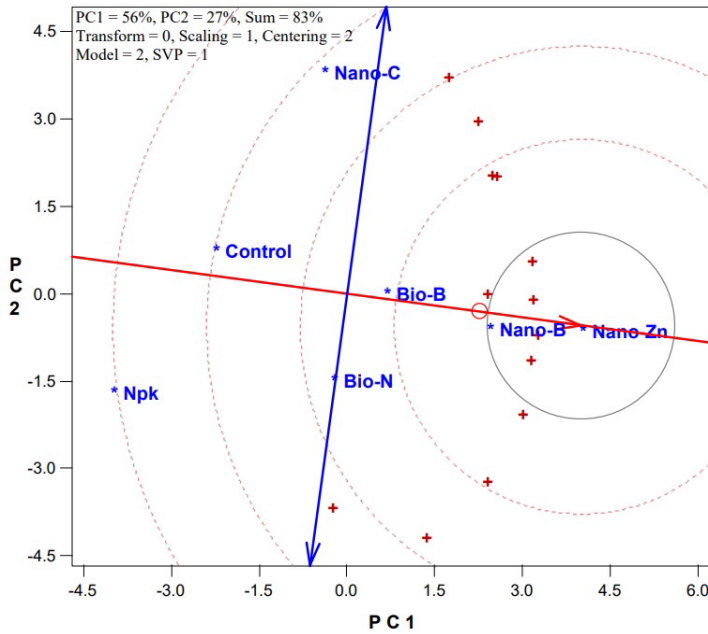


Figure 3. Ideal treatment tool of treatment by trait (TT) biplot.

For the quest for identifying an ideal treatment, it is generated as one that incorporates various favorable traits in its response. In Fig. 3, the single-arrow line represents the mean-trait axis, where fertilizers are ranked based on their trait response and divides this axis into two, with the right portion displaying fertilizers above mean and the left indicating those below mean (Mohammadi *et al.*, 2023). Based on this biplot (Fig. 3), Nano-Zn, Nano-B, and Bio-B exhibited above-average performance, while Bio-N, Nano-C, Control, and NPK (nitrogen + phosphorus + potassium) demonstrated below-average performance across traits.

The underperformance of NPK observed in this research aligns with the findings of Senthilkumar *et al.* (2021), who noted that micronutrient applications, such as zinc, outperformed NPK fertilizer. An ideal fertilizer should boast the high response for all traits, indicated by the long projection onto the mean trait axis and the short fertilizer vector. From Fig. 3, Nano-Zn and Nano-B fertilizer are close to the location of an ideal one, ranking as high regarding the response of traits due to their desirability across most traits. These treatments could serve as preferable alternatives to conventional fertilizers like NPK or other bulk fertilizers.

Current finding is supported by the study of Yousefzadeh *et al.* (2021), who highlighted that nano-forms of zinc and boron application yielded superior performance in various agronomic and yield traits of maize.

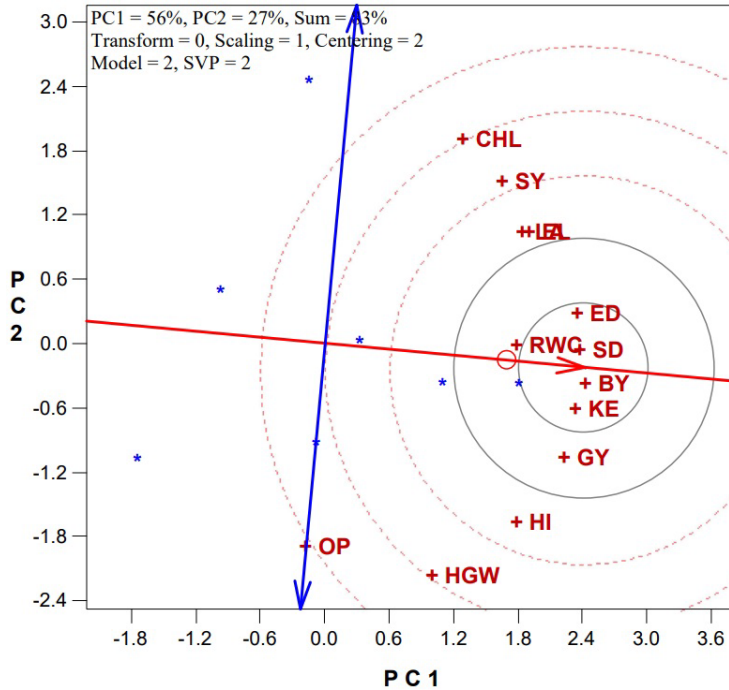


Figure 4. Ideal trait tool of treatment by trait (TT) biplot.

Traits are: leaf area (LA), kernels per ear (KE), chlorophyll content (CHL), ear diameter (ED), grain yield (GY), ear length (EL), stem diameter (SD), relative water content (RWC), biomass yield (BY), straw yield (SY), hundred grain weight (HGW), harvest index (HI) and oil percentage (OP).

In the TT biplot, the ideal trait incorporates various favorable treatments in its response. In Fig. 4, the single-arrow line represents the average-tester axis abscissa, where traits are ranked based on their response to treatments (Ebrahimi *et al.*, 2023). Based on this biplot (Fig. 4), biomass yield (BY) and stem diameter (SD) following to kernels per ear (KE), ear diameter (ED), relative water content (RWC) and grain yield (GY) exhibited similar properties like to ideal trait and indicated above-average performance, while oil percentage (OP), hundred grain weight (HGW), chlorophyll content (CHL) and straw yield (SY), demonstrated unfavorable performance across treatments. In the vector-tool of the model depicted in Fig. 5, treatments closely associated with the target trait of good grain yield

in maize are highlighted. Nano-Zn and Nano-B emerge as fertilizers good for achieving desirable yield performance, indicating that their usage is expected to enhance grain yield under drought stress conditions.

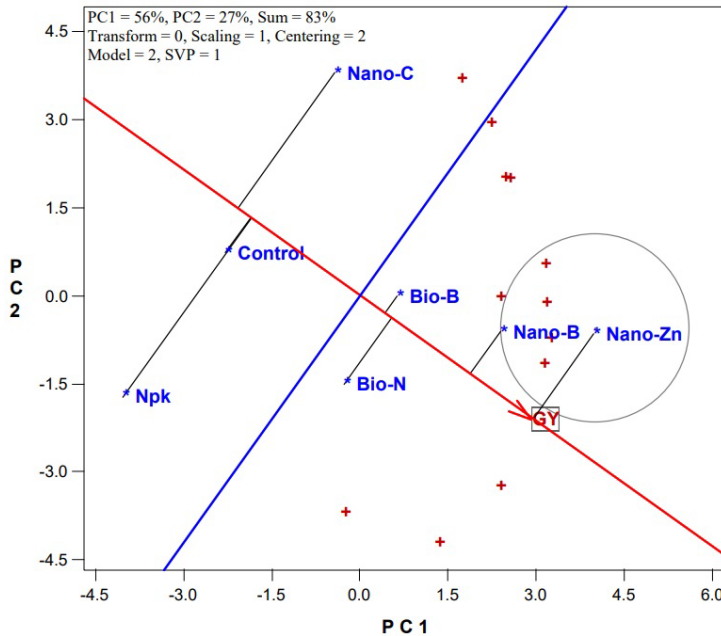


Figure 5. Examine a trait tool of treatment by trait (TT) biplot, for the selected trait (GY, grain yield).

This implies that the use of nano-sized micronutrient fertilizers not only promotes high yield performance but also improves other agronomic characteristics, thereby enhancing the widespread adoption of such fertilizers. Azam *et al.* (2020) demonstrated the positive effect of zinc nano-fertilizer usage in plant growth and performance of maize, attributed to increased activity of growth hormones. Similarly, Al-Juthery and Al-Maamouri (2020) emphasized the positive effects of boron nano-fertilizer in improving potato traits. Saritha *et al.* (2022) found that nano-composites were safe for crop products, suggesting that the use of nano-sized fertilizers is not only beneficial for crop production but also offers economic advantages.

In corn genotypes, two yield components [kernels per ear (KE) and hundred grain weight (HGW)] are crucial for selecting genotypes with superior grain yield (GY) performance. Fig. 6 presents a biplot based on these traits, which captures significant variability; the first two principal components (PCs) account for 85% and 15% of this variation, respectively. The trait vectors illustrate the

interrelationships among them, a pattern consistent across various datasets. Notably, grain yield is more closely related to the number of kernels per ear than to the hundred grain weight. Other studies have also highlighted the importance of the number of kernels per ear (Liu *et al.*, 2020; Sabitha *et al.*, 2024). For optimal selection of corn genotypes, it is advisable to prioritize kernels per ear (KE) over hundred grain weight (HGW) in the selection indices. Moreover, selecting based solely on both traits is not recommended due to their weak positive correlation; focusing on kernels per ear alone yields satisfactory results. The TT biplot model demonstrates that selecting based on kernels per ear during the early stages of selection is not only logical but also efficient. Additionally, this model facilitates genotype assessment based on these two traits. The predominance of the number of seeds per plant over seed weight in various cereals has been documented by numerous researchers (Matsuyama and Ookawa, 2020; Tehulie and Eskezia, 2021).

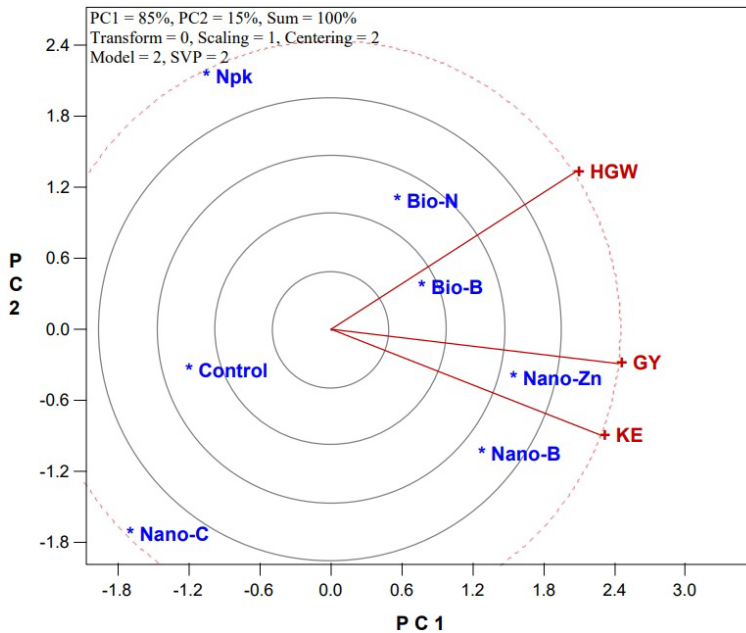


Figure 6. Vector tool of treatment by trait (TT) biplot, for kernels per ear (KE) and hundred grain weight (HGW) with grain yield (GY).

Studies have demonstrated that the utilization of nano-fertilizers leads to significant increased efficiency nutrient application, decreased toxicity in fields, mitigation of adverse impacts related with over-application, and reduced rates of usage. This is particularly significant for reaching the goals of sustainable farming, especially in undeveloped regions. The emergence of nanotechnology has introduced

a variety of nanomaterials with unique biologic as well as physical and chemical properties. Encapsulation of fertilizers within nanoparticles is one such innovation, which can be achieved through methods such as encapsulation inside nanopores, coating with thin polymer films, and delivery as emulsions of nano-scale dimensions (Konappa *et al.*, 2021). Nano-size fertilizers integrate nano-tools for synchronization the gradual delivery of fertilizers with crop uptake, thus preventing unfavorable fertilizer degradation in the field. The employed TT biplot model serves as an accomplished option for imaged analysis of dataset. In comparison to the routine numerical models, this procedure offers several benefits: (i) graphical presentation enhances understanding of dataset patterns, (ii) interpretative nature facilitates pairwise comparisons between treatments. However, a potential limitation of the biplot method is its failure to describe most of the variability in some conditions, thereby not displaying all structures of the data. Even in such instances, it can be ensured that this model of the PC1 and PC2 still captures the large portion significant data structures.

Conclusions

This study revealed notable disparities among various nano, bio, and bulk fertilizers concerning maize performance. Specifically, we found that two nano-fertilizers, namely nano-zinc and nano-boron, outperformed other fertilizers by enhancing the productivity of several key traits in maize, so their application emerges as a pivotal factor in maize production.

Conflict of interests. The authors declare that they have no conflict of interest.

Institutiunal Review Board and Ethics Committee Statement. Not applicable.

Informed Consent Statement. Not applicable.

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Authors contributions. Conceptualization (Janmohammadi, M.); methodology (Samadi, S.); investigation (Janmohammadi, M.); data curation (Sabaghnia, N.); software (Sabaghnia, N.); writing Sabaghnia, N.); supervision (Janmohammadi, M.); funding acquisition (Samadi, S.); All authors have read and agreed the published version of the manuscript.

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