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Zn-NANOBIOFERTILIZERS: GREEN SYNTHESIS AND THEIR PHYTOSTIMULATORY POTENTIAL

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Abstract

In agriculture sector, the unchecked extensive use of various agrochemicals to meet demand of high crop yield for growing population, adversely affects the soil and plant health; therefore, scientists are focusing on the development of new ecofriendly, effective and economic strategies for sustainable agriculture. In the present study, the most ancient concept of Biofertilizers and most recent concept of nanofertilizers was used to synthesize, Zn-Nanobiofertilizers by using consortia of Plant Growth Promoting Rhizobacteria and Zinc nanoparticles (zinc as micronutrient). For this purpose, consortium of gram positive Bacillus *bingmayogenesis* (KH3) and *Bacillus tropicus* (LS5) and gram-negative consortium of Achromobacter *denitrificans* (HS4) and *Pseudomonas* sp., (AH2) and green synthesized zinc nanoparticles by using leaf extracts of *Coriandrum sativum* and *Azadirachta indica* was used. The phytostimulatory impacts of prepared Zn-Nanobiofertilizers along with various individual treatments i.e., plant leaf extracts, PGPR consortia, zinc salt and nanoparticles were evaluated by selecting *Zea mays* L. as test crop under wirehouse conditions. The NBF application showed significant increment in shoot length, root length, number of leaves, fresh weight, protein content and chlorophyll content of treated plants as compared to control and all other treatments. Nano-biofertilizers (NBFs) improved plant health and yield by synergistic effect of PGPR and Zn-nanoparticles.

Index Terms: *Azadirachta Indica*, *Coriandrum Sativum,* Green Synthesis, Nanoparticles, Nanobiofertilizers, PGPR, *Zea Mays.*

1. INTRODUCTION

The excessive use of chemical fertilizers, causes degradation of agro-ecosystem and is a major source of environmental pollution that exert negative impact on plant and human health. These agro-chemicals cause destruction of biotic communities , biotic and abiotic stresses to plants and also affect the water holding capacity, soil fertility, salinity and nutrient balance of soil [1],[2]. So, it is the need of hour to develop effective, ecofriendly techniques for more agricultural productivity with minimum side effects. Biofertilizers are regarded as a substitute for chemical fertilizers, their application is a natural, nontoxic and ecofriendly strategy, which enhance soil nutrients balance required by the plant for optimal crop production[3].

Biofertilizers include beneficial microbes such as blue-green algae, fungal mycorrhiza, PGPR (plant growth promoting rhizospheric bacteria) and plant material that improve plant health and annual crop yield. Use of PGPR as biofertilizers has been suggested as more suitable approach, they are free living rhizospheric bacteria such as *Azotobacter*, *Azospirillium, Arthrobacter, Bacillus*, *Pseudomonas* and *Enterobacter etc*. which influence plant growth by various direct and indirect mechanisms such as phytohormone and siderophore production, inhibition of biofilm formation, supplying fixed nitrogen, soil moisture retention, supporting phosphorous uptake by solubilization of inorganic phosphates, prevention from phyto-pathogenic attack through synthesis of antimicrobial metabolites and volatile organic compounds. Application of PGPR as biofertilizers is a sustainable approach towards agricultural sustainability [4], [5].

But use of biofertilizers also have some limitations such as decreased performance in fluctuating environment, high dosage requirement and shelf life. Therefore, Nanotechnology is gaining more attention in agriculture sector as latest more effective technique, currently it thought to be the foundation for numerous innovations especially in agricultural science. Generally, it deals with nanoparticles ranging in size from 1 to 100 nm with unique biological, physical and chemical properties which enable innovative interactions with intricate biological processes due to large surface-to-volume ratio, compact size and their multifunctionality.

Nanoparticles help to deal with factors that affect plant health and growth, they enable the effectiveness of chemicals with their low dosage leads to alleviate environmental stresses caused by chemical fertilizers in agriculture[6]. And this can be achieved more effectively by using Green Synthesized nanoparticles. Plants have the ability to synthesize metals and metal oxide nanoparticles with unique properties due to their biochemical components. The biological method for synthesis of nano-materials by using environment friendly ingredients that are sustainable, quick, economical and non-toxic in nature is known as Green Synthesis [3].

To enhance the chances of crop establishment, scientists have formulated Nano-biofertilizer. Nano-biofertilizers is a combined formulation of nanofertilizer and biofertilizer, having dual characteristics of bio-inoculants and nanoparticles. It is more effective than other traditional agricultural strategies, and provides essential plant nutrients due to nanomaterial and microbial revitalization. The characteristics of nano-biofertilizers includes high efficiency, ease to handling, low quantity usage, and cost effectiveness, eco-safety nature.

Nanobiofertilizers perform more efficiently than other traditionally used agricultural salts and improve plant health and crop yields by synergistic mechanisms of nanofertilizer and biofertilizer with intensified responses. Nanobiofertilizers enhance the plant growth and quality by the availability of nutrients to plant by maintaining the dissolution and quick uptake of nano-size particles of fertilizer, by regulating metabolic processes such as production of secondary metabolites, performing bioremediation etc. [7],[8].

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The present research work encircles the green synthesis of zinc- nanobiofertilizers to check their phytostimulatory impact on *Zea mays* L. *Zea mays* L. is an annual crop that ranks third in the world after rice and wheat in terms of cereal crops. However, the yield potential and nutrient content of maize are reducing because of biotic and abiotic challenges, such as deficiency of micronutrients in the soil, particularly zinc. Zinc is the trace element which is vital for healthy growth and reproduction cycle of plants as it plays crucial functions in controlling growth activating enzymes, regulating gene expression, activating phytohormones, synthesizing proteins and metabolizing carbohydrates. The Zn deficiency also reduces the nutritious value of cereal grains [9], [10], [11].

Nano-dimensional zinc oxide particles improve plant growth, pigmentation, protein content, sugar and zinc elevated antioxidant activity of enzyme. So, Green synthesis of nanoparticles is the major source of production of ZnNPs, *because of their phytochemicals which causes the reduction of metal salts into their nanoparticles* [12]. So, in current work, the nanoparticles of zinc were synthesized by using *Azadirachta indica (Neem) and Coriandrum sativum (Dhania) leaf extracts* and their synergistic impact was observed by combined application of zinc nanoparticles with gram positive and gram-negative PGPR consortia individually, as zinc nanobiofertilizers.

The growth promoting impact of prepared zinc nanobiofertilizers along with other treatments i.e., leaf extracts (NE, CE), Zn-nanoparticles (N-ZnNPs, C-ZnNPs), PGPR consortia (P+, P-) and Zinc salt (S) were estimated by selecting *Zea mays* L. as test crop under wirehouse conditions by evaluating the growth and biochemical parameters of treated and nontreated plants.

2. MATERIALS AND METHODS

2.1 Green Synthesis of Zinc Nanoparticles

The fresh leaf extracts of *Azadirachta indica* (Neem) and *Coriandrum sativum* (Coriander) were prepared by following Shah et al. [13]. For this purpose, ten grams of crushed fresh leaves of each plant were taken and boiled in 100ml of distilled water separately, and filtered through Whatman filter paper no. 01. The resulting filtrates (leaf extracts) were stored in refrigerator for further use. After this, Zinc nanoparticles were synthesized by using Zinc Sulfate ($ZnSO_4.7H_2O$) with leaf extracts by following Selim et al. [14] with slight modifications. For synthesis, 25 ml leaf extract of *A. indica* and *C. sativum* was taken separately and then 2.5 grams of Zinc sulfate in each pre-heated extract was added and further heated until the formation of yellowish creamy precipitates. These precipitates were air dried and purified to obtain Zn nanoparticles (abbreviated as N-ZnNPs using neem extract and C-ZnNPs by using coriander extract).

2.2 Characterization of Zinc Nanoparticles

UV-visible spectroscopic analysis:

Initially, Zinc nanoparticles were analyzed by measuring absorbance at 200-600 nm by using UV-visible spectrophotometer.

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Fourier transformer infrared (FTIR) spectroscopic analysis:

The prepared Zinc nanoparticles were also analyzed by using Fourier transformer infrared (FTIR) spectroscopy.

2.3 Preparation of PGPR consortia

In this study, four already isolated and identified PGPR strains were taken from Microbiology Research Laboratory, Institute of Botany, University of the Punjab Lahore. Then, PGPR consortia i.e., gram-negative consortium consisting of *Pseudomonas* sp., (AH2) and *Achromobacter denitrificans* (HS4) and gram-positive consortium comprising of *Bacillus bingmayogenesis* (KH3) and *Bacillus tropicus* (LS5), were prepared by incubating PGPR strains in L-broth growth medium at 37^oC for 24 hours and then by mixing the equal amount of respective bacterial cultures. The prepared consortia were centrifuged at 6000 rpm, supernatant was discarded and obtained pellets of each PGPR consortium were re-dissolved in distilled autoclaved water for further use.

2.4 Synthesis of Zinc Nanobiofertilizers

Nano-biofertilizers (NBFs) were prepared by mixing the synthesized Zn nanoparticles (N-ZnNPs and C-ZnNPs) and PGPR consortia (P+ & P-) by following Akhtar et al. (2022) [15] and Sharma et al. (2023)[16] with slight modifications. For this synthesis, the solutions of both N-ZnNPs and C-ZnNPs were formed (0.05g/100ml) separately then 45ml of 0.05% solution of N-ZnNPs and C-ZnNPs were mixed with 5ml of both PGPR consortia (gram positive P+, gram negative P-) separately.

The nanobiofertilizers synthesized by using neem extract-based N-ZnNPs and positive and negative PGPR consortium were represented by N-NBF+, N-NBF- respectively, and nanobiofertilizers synthesized by using coriander extract based C-ZnNPs with gram-positive and gram-negative consortium were represented by C-NBF+ and C-NBF- respectively.

2.5 Plant Nanobiofertilizers (NBF) Interaction Assay

For the experimental setup, the certified seeds of test crop of *Zea mays* L. (var. MMR-1) were obtained from Federal Seed Certification and Registration Department, Lahore. Before sowing seeds were surface sterilized, using 0.1% mercuric chloride solution for 40 second and then washed 7-8 times with autoclaved distilled water.

To determine the growth promoting effect of various treatments, the experiment was designed in randomized complete block design (RCBD) under wire house conditions and different treatments were applied including Neem leaf extract (NE), Coriander leaf extract (CE), Neem based zinc nanoparticles (N-ZnNPs), Coriander based zinc nanoparticles (C-ZnNPs), Zinc salt (S), gram-positive consortium of PGPR (P+), gram-negative consortium of PGPR (P-) and nanobiofertilizers (N-NBF+, N-NBF-, C-NBF+ and C-NBF-) in comparison to control which was without any treatment.

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The plants were harvested after 25 days of growth and the impact of different treatments were estimated by observing the growth parameters (fresh weight, root length, shoot length and number of leaves) and biochemical parameters i.e., protein content [17] and chlorophyll content [18] of treated plants as compared to control.

2.6 Statistical Analysis

Statistical analysis of obtained data of growth and biochemical parameters was done with SPSS software by using Duncan's Multiple Range Test.

3. RESULTS

3.1 Green synthesis of Zinc Nanoparticles

The presence of secondary metabolites in leaf extracts caused the formation of yellowish white precipitates as zinc nanoparticles due to oxidation reduction activity. Both leaf extracts were found to be effective to synthesize zinc nanoparticles due to their bio-reductant metabolites.

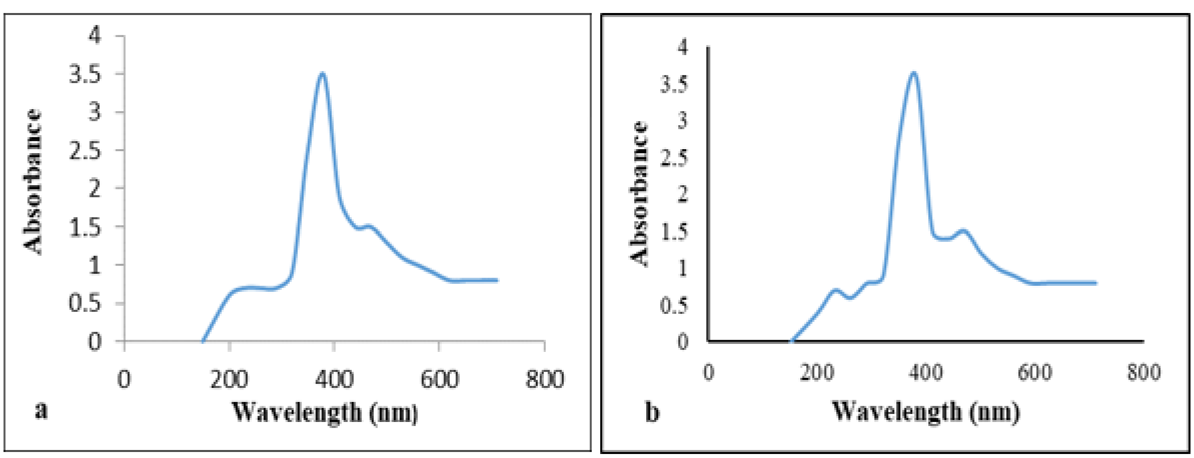
3.2 Characterization of Zinc Nanoparticles

UV-visible spectroscopic analysis:

The formation of Zn nanoparticles by two different leaf extracts was analyzed by UV-vis spectroscopy, which showed absorption peak at 350- 380 nm range (Fig. 1 a & b).

Fourier transformer infrared (FTIR) Spectroscopic analysis:

In case of N-ZnNPs, FTIR spectrum showed -O-H band at 3199cm^{-1}, C-O stretching bands at 1064 cm^{-1} and -C-H bending vibration of secondary alcohol and benzene at 848 cm^{-1}. In case of C-ZnNPs, the spectrum showed the absorption band of –O-H stretching vibration of alcohol and phenol groups at 3200 cm^{-1}, –C-O stretching vibration at 1073 cm^{-1} and –C-H bending vibration at 1017 cm^{-1}, which indicated the presence of alkane and primary alcohol respectively (Fig 1 c & d).



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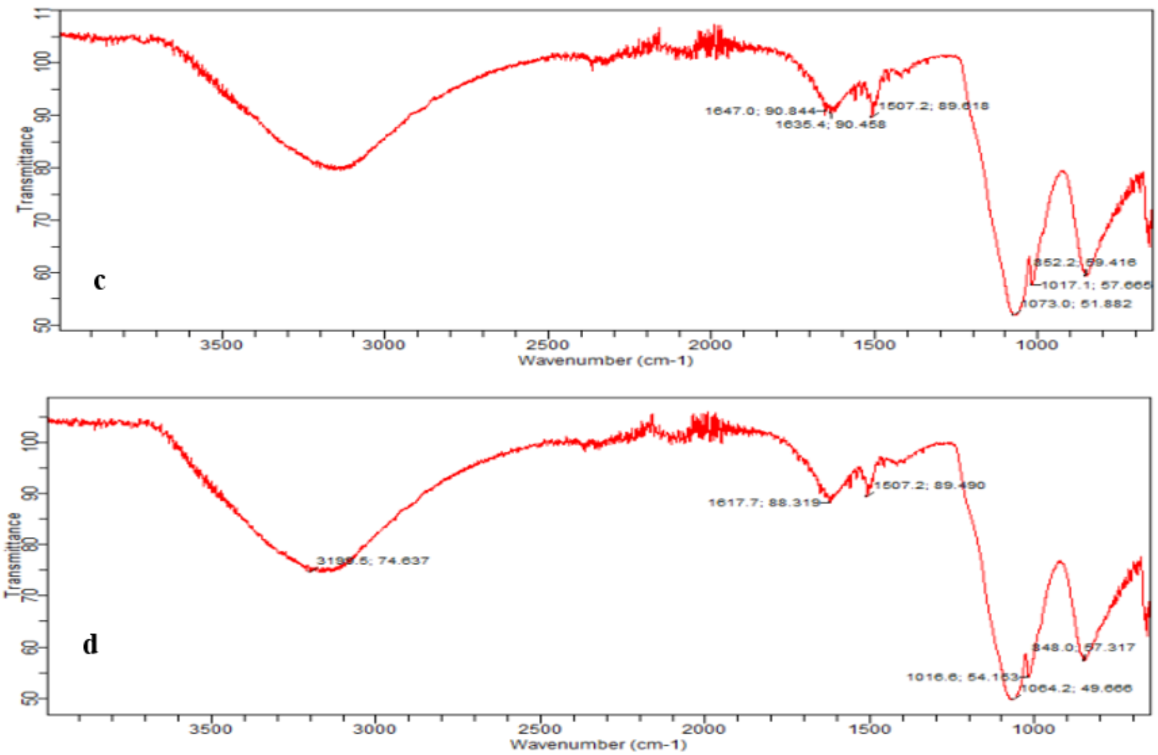

Fig 1: (a) UV-Vis spectrum of Neem extract based ZnNPs (N-ZnNPs) (b) UV-Vis spectrum of Coriander extract based ZnNPs (C-ZnNPs) (c) FTIR spectrum of Neem extract based ZnNPs (N-ZnNPs), (d) FTIR spectrum of Coriander extract based ZnNPs (C-ZnNPs) Coriander extract based ZnNPs

3.3 Preparation of PGPR Consortia

Two PGPR consortia were prepared i.e, consortium of gram-positive *Bacillus bingmayogenesis* (KH3) and *Bacillus tropicus* (LS5) and gram-negative consortium of *Pseudomonas* sp., (AH2) and *Achromobacter denitrificans* (HS4) with same O.D to evaluate the effectiveness of both consortia in nanobiofertilizers.

3.4 Plant Nanobiofertilizers (NBF) Interaction Assay

The phytostimulatory impact of nanobiofertilizers was determined by measuring the growth parameters and biochemical analysis of treated plants as compared to control.

3.4.1. Growth Parameters

The effects of different treatments on growth enhancement of test crop are given below.

Shoot length

For growth parameters, in case of shoot length, the treatment of leaf extract (CE extract) promoted growth significantly with increment of 17% than neem extract (NE) with 15% increment. For PGPR consortia, gram-negative consortium (P-) showed better results

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with 8% increment in shoot length than gram-positive consortium (P+) with 6.7% increment. The treatment of plants with zinc salt (S) showed only 5.6% increment, which increased upto 14% for N-ZnNPs and 8.3% increment for C-ZnNPs. Overall, the treatments with NBFs showed significant results among all treatments. The neem based nanobiofertilizers having gram positive consortium (N-NBF+) showed maximum 33% increment of shoot length in treated plants followed by coriander based nanobiofertilizer having gram-negative consortium (C-NBF-) with 22.9%, coriander based nanobiofertilizer having gram-positive consortium (C-NBF+) with 22.5% and neem based nanobiofertilizer having gram-negative consortium (N-NBF-) with only 2% increment as compared to control (Fig2, a). The combined application of PGPR and ZnNPs (NBFs) showed more significant results than other individual treatments of PGPR consortia (P+, P-) and zinc nanoparticles (N-ZnNPs, C-ZnNPs), zinc salt (S) and leaf extracts (CE, NE).

Root length

In case of root length, the treatment of plants with leaf extracts, NE treatment showed significant increment of 7.3% in root length than CE with 0.2% increment. The gram-positive consortium (P+) showed better results with 3% increment than gram-negative consortium (P-) with 0.12% increment. The treatment of zinc salt (S) showed only 1.18% increment while the treatment of N-ZnNPs showed 8.4% and C-ZnNPs recorded 1.88% increment. Overall, the treatment of plants with NBF showed more significant results among all treatments. The neem based nanobiofertilizer having gram-positive consortium (N-NBF+) showed maximum increment of 20.97% in root length followed by coriander based nanobiofertilizer having gram-negative consortium (C-NBF-) with 19.9%, coriander based nanobiofertilizer having gram-positive consortium (C-NBF+) with 15.6% and neem based nanobiofertilizer having gram-negative consortium (N-NBF-) with only 11% increment in root length as compared to control (Fig 2. a).

Number of leaves

In case of number of leaves, the treatment of plants with leaf extract CE treatment promoted plant growth significantly with 5.7% increment in number of leaves than the leaf extract of *A*. *indica* (NE) which showed 3.4% increment in number of leaves. The treatment of gram-negative consortium (P-) showed better results with 6.7% increment than gram-positive consortium (P+) which showed 3.4% increment. The treatment on zinc salt (S) showed 8% increment and neem-based zinc nanoparticles (N-ZnNPs) treatment showed 8.4% increment than coriander-based zinc nanoparticles (C-ZnNPs) treatment which showed 5.7% increment. Among the treatments of nanobiofertilizers, neem based nanobiofertilizer with gram-positive consortium (N-NBF+) showed maximum number of leaves with 11.7% increment as compared to control followed by the 6.7% increment in coriander based nanobiofertilizer having gram-positive consortium (C-NBF+) and coriander based nanobiofertilizer having gram-negative consortium (C-NBF-) treatments and then 5.1% increment in N-NBF- treated plants as compared to control (Fig 2. b).

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Fresh weight

The treatment of plants with leaf extracts the (CE) showed 9.1% increment in the fresh weight of plants than NE with only 0.41% increment as compared to control. The treatment of gram-negative consortium (P-) showed 6.9% increment than gram-positive consortium (P+) with 5.1% increment. The treatment of zinc salt (S) showed 4.1% increment while the neem-based nanoparticles (N-ZnNPs) showed significant results with 24% increment than coriander-based nanoparticles (C-ZnNPs) which showed only 5.7% increment in fresh weight. The treatment of plants with neem based nanobiofertilizer having gram-positive consortium (N-NBF+) showed maximum increment of 35% in fresh weight followed by coriander based nanobiofertilizer having gram-positive consortium (C-NBF+), coriander based nanobiofertilizer having gram-negative consortium (C-NBF-) and neem based nanobiofertilizer having gram-negative consortium (N-NBF-) with 22.3%, 22.1% and 5.1% increment in fresh weight respectively as compared to control (Fig 2. b).

3.4.2. Biochemical parameters

The effects of different treatments on the biochemical parameters of test crop are given below.

Protein content

For biochemical parameters, in case of protein content, the treatment of plants with leaf extract, NE treatment showed more significant increment of 201% than CE treatment which showed 190% increment as compared to control. The gram-positive consortium (P+) treatment to plants showed 198% increment while gram-negative consortium (P-) with 188% increment. The treatment of zinc salt showed 190% increment, while the treatment of neem-based nanoparticles (N-ZnNPs) showed 203% increment than the coriander based (C-ZnNPs) which showed 176% increment in protein content. The treatment of coriander based nanobiofertilizer having gram-positive consortium (C-NBF+) showed maximum increment of 239% followed by the coriander based nanobiofertilizer having gram-negative consortium (C-NBF-) treatment with 206%, neem based nanobiofertilizer having gram-positive consortium (N-NBF+) with 198% and neem based nanobiofertilizer having gram-negative consortium (N-NBF-) with 185% increment in protein content (Fig 2. c).

Chlorophyll content

In case of chlorophyll content treatment of plants with coriander extract (CE) showed 26.2% increment in chlorophyll content as compared to control and NE treatment showed 13.7% increment. The treatment of plants with gram-positive consortium (P+) showed 36.5% increment than gram-negative consortium (P-) with 14.5% increment. The treatment of zinc salt showed 15.3% increment in chlorophyll content while the neem-based nanoparticles (N-ZnNPs) showed 13.7% increment than the treatment of coriander-based nanoparticles (C-ZnNPs) which showed 13.2% increment. The coriander based nanobiofertilizer having gram-positive consortium (C-NBF+) treatment showed 35% increment followed by neem based nanobiofertilizer having gram-negative

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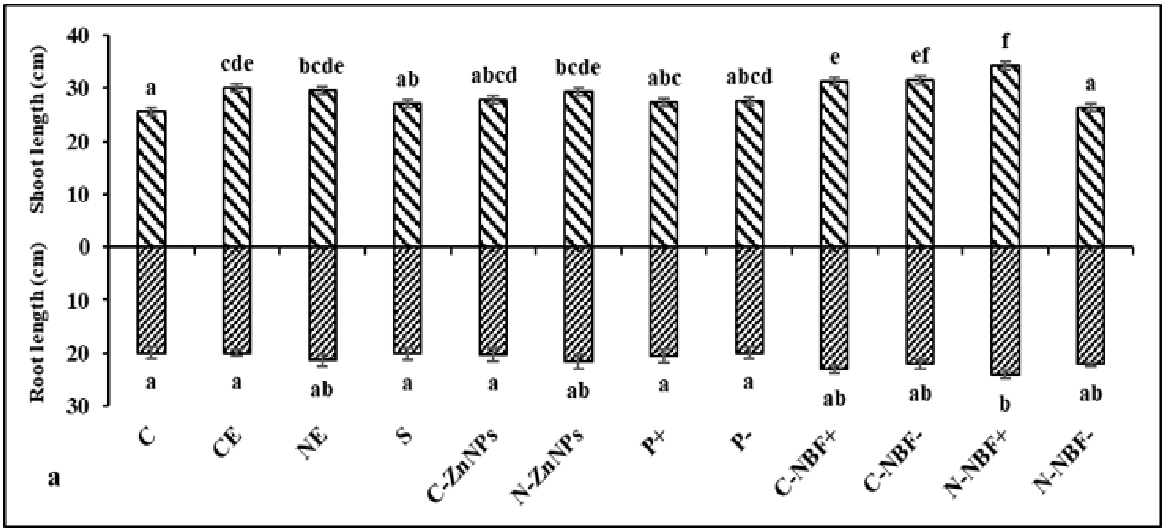
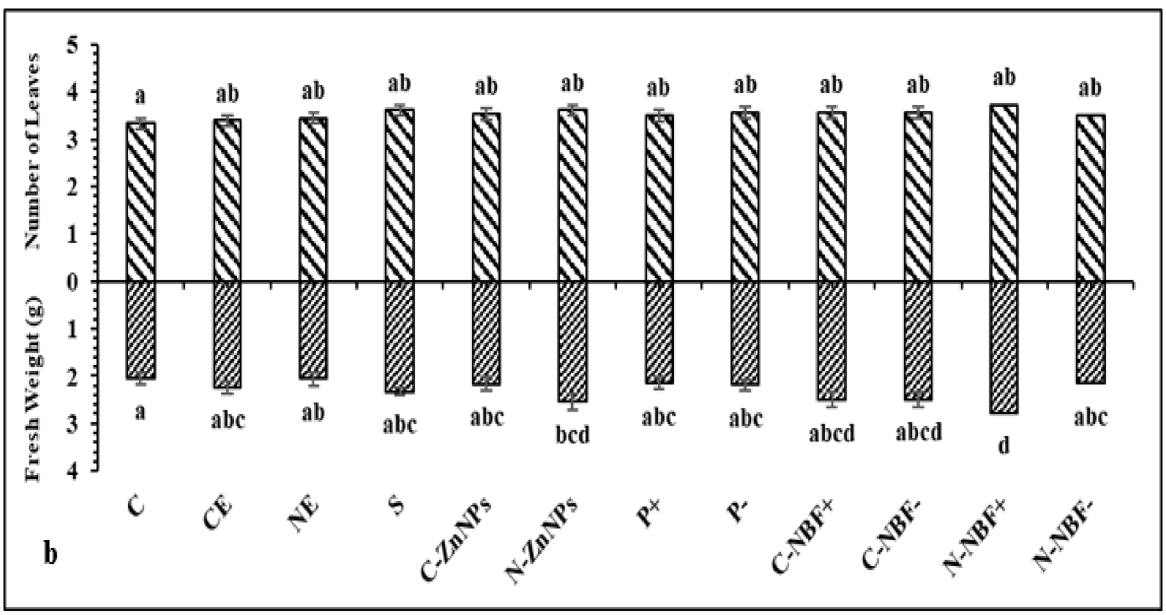
consortium (N-NBF-), coriander based nanobiofertilizer having gram-negative consortium (C-NBF-) and neem based nanobiofertilizer having gram-positive consortium (N-NBF+) exhibiting 25.3%, 19.8% and 17.6% increment in chlorophyll content as compared to control (Fig 2. c).





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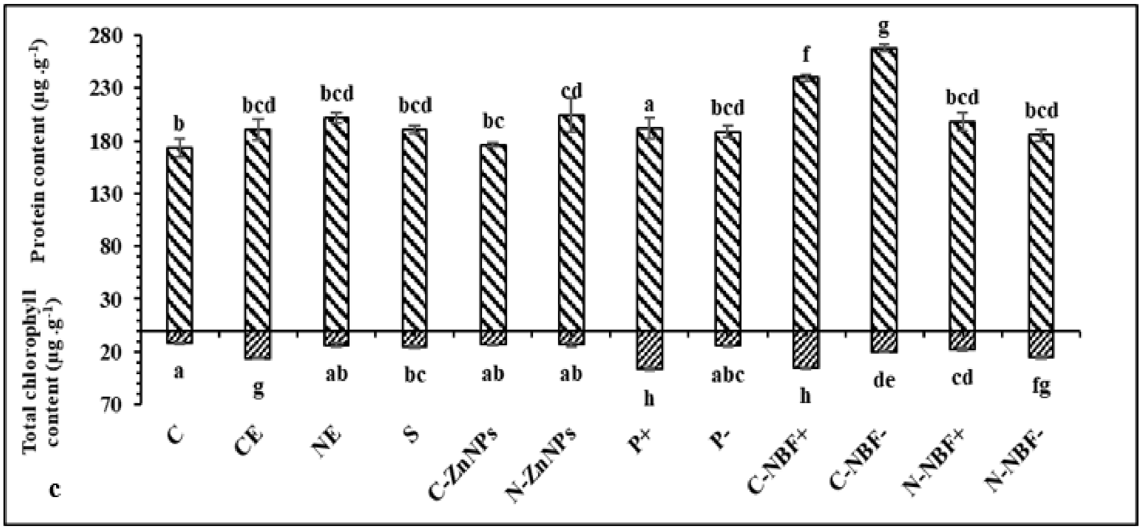

Fig 2: Impacts of different treatments [zinc sulfate (S), leaf extracts of Neem (NE) and Coriander (CE), zinc nanoparticles (C-ZnNPs, N-ZnNPs), PGPRs consortia (P+, P-) and Nanobiofertilizers [C-NBF (+, -), N-NBF (+, -)] on growth and biochemical parameters of *Zea mays* L (a) impact of treatments on shoot length and root length (b) impact of different treatments on fresh weight and number of leaves (c) impact of different treatments on Protein content and Total chlorophyll content of *Zea mays*

4. DISCUSSIONS

Modern agricultural practices rely extensively on chemical fertilizers but their long-term use is potentially harmful to the environment, nutritional stability, soil fertility and rhizospheric microbial community. Plant development and stress tolerance can be improved by combined application of biofertilizers and nanofertilizers, it acts as additional tool regarding environmental sustainability which is highly adaptable, and long-lasting. Nanobiofertilizers cause significant enhancement in plant growth by the reduction of abiotic and biotic stresses (such as salinity, drought, flooding etc.), promotion of nutrients solubilization and nutrients availability, production of phytohormones (gibberellins and auxins) and plant resistance to diseases. These effects can be achieved more efficiently by Green synthesis of nanomaterial, because green synthesis by using plants is an eco-friendly strategy, the chemical components present in plants, these components are responsible for the reduction of metal ions into nanostructure [19], [20]. A wide range of water-soluble plant metabolites i.e., ketones, flavones, aldehydes, organic acids and amides present in *Azadirachta indica* (Neem)and *Coriandrum sativum* (Coriander), leaf extract act as bio-reductants for the rapid reduction of Zn ions into nanoparticles. The presence of these naturally occurring flavonoids, terpenoids, reducing sugars, several protein molecules and enzymes in leaf extracts assist in bio-reduction and stabilization of biosynthesized Zn nanoparticles [21]. The use of nanobiofertilizers in agriculture is still at

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early stages, but has potential to turn traditional techniques of agriculture into advance agriculture more quickly than any other existing strategy [16].

Treatment of nanobiofertilizers caused significant increase in shoot length of treated plants, the NBF showed maximum increment of 33% due to the availability of Zn nutrient in the form of Zn nanoparticles and improvement in the nutritional status of the soil due to presence of PGPR [15], [22]. There are various mechanisms of PGPR such as nitrogen fixation, siderophores production and production of plant growth hormones and secondary metabolites etc. which are involved in plant growth promotion. Nanoparticles readily uptake by plant, incorporate in plant body and regulate different enzymatic actions and complete growth cycle of plant to promote plant growth and size [23]. The application of NBF showed 20.97% increment in root length. The significant increase in root length and root hair directly influences the plant growth, the rooting of plant enhance due to the production of rooting hormones such as auxins (IAA, IBA, GA3), root structural modification and mineral solubilization. The auxin producing PGPR help in roots development by using mechanisms of plant growth [24]. The application of Zn nanobiofertilizers significantly improved the root length, root dry weight and root surface area because zinc present in the form of nanoparticles regulates the biochemical pathway of auxin production to improve root health and development and various metabolic and enzyme activities and biochemical processes [25]. The application of zinc nanobiofertilizer causes the opening and closing of stomata, improve anatomy, function, leaf number and boosts the photosynthetic rate, thus in current study, it also enhanced the number of leaves of treated plants. Therefore, NBF treatment showed 11.7% increment in number of leaves [16], [26]. The application of zinc nanobiofertilizers increase the fresh weight, N-NBF treatment showed maximum 35% of increment. This growth and productivity of plants, improved nutrient absorption and translocation capability, higher rate of photosynthesis and the storage of photosynthate, increased production of proline, production of biomass and decreased leakage of electrolytes is due to PGPR and zinc nanoparticles. Zinc is directly involved in carbohydrate metabolism which leads to fresh weight increment [27], [28].

For biochemical analysis, NBF treatment showed 239% increment of protein content, the application of Zn nanoparticles in the form of Zn-NBF improve protein content and yield of grains. Zinc is a micronutrient act as a co-factor and directly involved in regulation of DNA transcription and enzymatic processes necessary in protein metabolism [29]. The *Pseudomonas* sp. of PGPR causes increase in protein content of *Zea mays* as they regulate nitrogen fixation by nodule formation, convert inorganic nitrogen into organic nitrogen during protein synthesis and nutrient solubilization increase the protein content of plants. The application of NBF caused 35% increment in chlorophyll content. PGPR ensure the mineral solubilization and nutrient bio- availability to plants, it leads to stress reduction and improved plant health. Zn nanoparticles improve the anatomical structure of leaf and stem that influence the quantity of grana, resulting in enhancement of total chlorophyll content and carotenoids in leaves and greater photosynthetic capability of plants and enhance plant growth [30], [31].

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5. CONCLUSIONS

The use of nanobiofertilizers have potential to turn current agriculture system into smart agriculture and reduce harmful effects of agrochemicals on plants, human health and environment. Nanobiofertilizers enhance the plant growth and crop products more efficiently by regulating different mechanisms with minimum chance of toxicity due to synergistic qualities of biofertilizers and nanofertilizers. In the current research work, all the treatments showed significant results regarding growth promotion in *Zea mays*. The individual treatment of *C. sativum* (Coriander) leaf extract was more effective than leaf extract of *A. indica* (Neem), while in case of PGPR consortia, the gram-negative (P-) consortium showed more significant results in growth parameters and gram-positive (P+) consortium performed well in enhancement of biochemical parameters. The treatment of Zinc salt (S) showed significant results while the neem extract-based Zinc nanoparticles (N-ZnNPs) worked more efficiently in improvement of all parameters. Overall, the application of zinc nanobiofertilizers showed more significant results among all other treatments and increase growth as well as biochemical parameters of *Zea mays*. The Neem based nanobiofertilizers worked more significantly with P+ consortium (N-NBF+) while coriander extract based NBFs worked effectively with both P+ and P- consortium. Thus, nanobiofertilizers worked better than individual salt, plant extracts and even PGPR and nanoparticles. Using nanobiofertilizers is an efficient, effective and ecofriendly technique for sustainable agriculture as compared to traditional agricultural practices.

Table 1

List of Abbreviations

Abbreviations	Description
PGPR	Plant Growth Promoting Rhizobacteria
NBF	Nano-biofertilizer
ZnNPs	Zinc Nanoparticles
C	Control
CE	Leaf Extract of Coriander
NE	Leaf Extract of Neem
S	Zinc Salt
C-ZnNPs	Zn-Nanoparticles synthesized by using leaf extract of Coriander
N-ZnNPs	Zn-Nanoparticles synthesized by using leaf extract of Neem
P+	Consortium of Gram Positive PGPR
P-	Consortium of Gram Negative PGPR
C-NBF+	Nanobiofertilizer synthesized by using gram positive PGPR consortium + Coriander extract based Zn-nanoparticles
C-NBF-	Nanobiofertilizer synthesized by using gram negative PGPR consortium + Coriander extract based Zn-nanoparticles
N-NBF+	Nanobiofertilizer synthesized by using gram positive PGPR consortium + Neem extract based Zn-nanoparticles
N-NBF-	Nanobiofertilizer synthesized by using gram negative PGPR consortium + Neem extract based Zn-nanoparticles

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