[Now published in full after the initial publication as an Epub ahead of print August 8, 2022 at www.fluorideresearch.online/epub/files/200.pdf]

EVALUATION OF THE CURATIVE EFFECTS OF ZINC OXIDE NANOPARTICLES ON *SOLANUM MELONGENA* L. UNDER FLUORIDE STRESS

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ABSTRACT: Salinity is a major abiotic environmental factor around the world that affects plant growth deleteriously. A pot experiment of purple variety of *Solanum melongena* L. (brinjal) was conducted during the growth season of 2021 with completely randomized block design. Different treatments of sodium fluoride (NaF) (300ppm and 400ppm) revealed that NaF showed harmful effects on plant height, shoot length and root length of brinjal. It also decreased no. of leaves, leaf area, and no. of branches along with yield parameters and biochemical attributes. Crop production can be improved by using nanotechnology under fluoride stress. The reduction in growth parameters caused by NaF can be ameliorated by treating the plants with different concentrations of zinc oxide nanoparticles (ZnONPs) (100ppm, 200ppm, 300ppm, 400ppm, and 500ppm) using soil drench and foliar spray methods. The ZnOPs were synthesized from java palm (*Syzygium cumini* L.) utilizing the green synthesis method. Exogenous application of ZnOPs enhanced growth parameters of brinjal plants under NaF stress. The upsurge level of chlorophyll a, chlorophyll b, total chlorophyll content, and carotenoids decline the toxic effect of NaF. The ZnOPs concentration at 500ppm increased biomass production at vegetative and reproductive stages.

Keyword: Brinjal, Chlorophyll Content Growth Parameters, Sodium Fluoride, Zinc Oxide nanoparticles

INTRODUCTION

Environmental factors including both biotic and abiotic regulate food production in the tropics. The floods, salinity droughts, high and low temperatures, and air pollution are among the most common abiotic stresses caused by a variety of environmental conditions [1]. Salinity is the most common abiotic stress, and it has a negative impact on plant growth and development all around the world [2]. Because of improper watering, saline zones continue to grow in size [3]. Salinity inhibits crop development and growth through a complicated set of features that includes mineral deficiencies, ion toxicity, osmotic stress, and physiological and biochemical abnormalities [4]. Fluorine is one of the most reactive elements which does not exist in its natural state. It is commonly found as fluoride (F) in environment and the total amount of fluoride in the Earth's crust is believed to be 0.3 g kg^{-1}. [5] Natural sources of fluoride include volcanic eruptions, marine aerosols and minerals, which lead to fluoride accumulation owing to weathering and leaching [6][7]. The element F is considered most harmful elements for plant [8][9], because it is not required for plant growth and various plant species are fluoride-sensitive [10] [11]. Different

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concentrations of NaF was applied on pea plant that cause toxic effect on plant height, biochemical process and on biomass production due to gradual accumulation of NaF in plant tissues [12]. Irrigation water contaminated with fluoride also has adverse effect on germination of seed and growth [13].

Nanotechnology is considered to be the advanced technique that has been identified as a potential technology for improving the agriculture production that plays an important role in livelihood of the poor. It also plays vital role in protection of plant, crop improvement, production, processing and security of food [14]. It also has not only the potential to improve plant growth, nutrient uptake and resistance from diseases but also improve the quality of yield. The product based on nanotechnology including nanopesticide, nanosensors, nanofertilizers and nano weedicides improves the crop production and farmer's income [15].The Greek noun "Nano" which means dwarf or short, the particle size of nanoparticles (NP) ranges from 5–250nm [16].

The synthesis of nanoparticles is expected to be a dynamic topic in nanoparticle research and application. There are a variety of ways to make nanoparticles, including these three methods: physical, chemical, and biological [17]. Zinc (Zn) is a vital nutrient that is required by all living organisms for their development. It is ranked as the 23rd most plentiful element on Earth [18] and the second most abundant transition metal [19], following iron. Zinc is a micronutrient that crops require for growth and development. Zinc also aids in the formation of chlorophyll by boosting the energy source [20]. Biological applications of Zinc oxide nanoparticles (ZnO-NPs) are considered as most significant nanoparticle because of its valuable effect on applied plants [21]. It enhanced the rate of seed germination, chlorophyll pigment in leaf and enhancement in production quality and quantity [22]. The main objective of this experiment was to investigate the stress induced by different concentrations of sodium fluoride as soil drench and foliar spray method on growth parameters, biomass and on photosynthetic pigments of *Solanum melongena* L. Furthermore, the research was intended to reveal the possible beneficial role of biologically synthesized ZnOPs on the growth, physiology, and yield of *Solanum melongena* L grown in NaF stress.

MATERIALS AND METHODS

The research was carried out in a botanical garden on the Quaid-e-Azam campus of the University of Punjab, Lahore, Pakistan. To improve soil fertility, sandy and loamy soil were mixed in a 3:1 ratio with leaf manure and farmyard, as well as DAP. This soil's composition made it ideal for promoting plant growth. The clay pots used in the experiment had a length of 12 inches and a diameter of 9 inches and were filled with a measured amount of 10kg of soil. According to the techniques of treatment and the amount of treatments, all of the pots were placed in a randomized complete block design (RCBD). The seeds of *Solanum melongena* L. (brinjal) was procured from Roshan seed center, Lahore, Pakistan. The healthy seeds were soaked in water overnight. Five seeds were sown in each pot. The seedling thinning was completed on February 15th and one seedlings were allowed to grow in every pot. Sodium fluoride solution (300ppm and 400ppm) was prepared by using measured amounts of NaF and dissolved in distilled water.

The leaf extract of *Syzigium cumini* L.which was taken from the botanical garden of the University of Punjab, Lahore, was used to synthesize ZnOPs. To make the extract, 20g of leaf powder was combined with 200ml distilled water in a beaker and set on a hot plate for 15 minutes. The filtration was done with Whatman's No. 1 filter paper. To make the solution, 0.16g of ZnSO4 was dissolved in 100ml distilled water following an equal volume of salt solution and leaf extract were added. For NP purification, 1ml of solution was poured in each Eppendorf after 4 hours and centrifuged for 15 minutes at 10000rpm. Sodium fluoride (300ppm and 400ppm) separately and along with ZnOPs (100ppm, 200ppm, 300ppm, 400ppm and 500ppm) were applied as soil drench and foliar spray methods. Each treatment has 3 replicates.

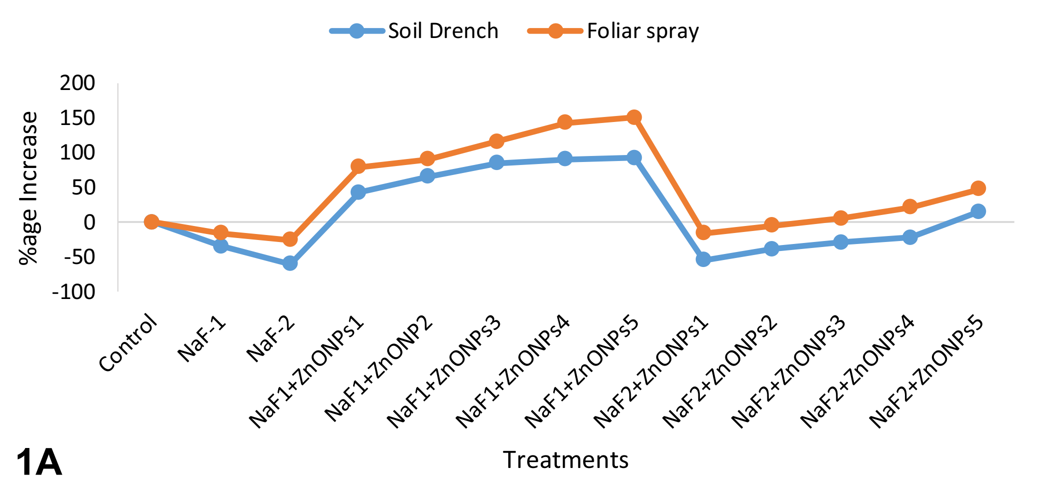
Sodium fluoride was applied twice a week and ZnOPs at 15-day interval: twice before vegetative harvest and twice before final harvest. 100ml NaF applied as a soil drench and 10ml applied as a foliar spray ZnOPs were applied at 100ml as a soil drench and 50ml as a foliar spray. The vegetative harvest was taken at 60 days after sowing (DAS). Plants uprooted from pots and were taken to the laboratory in plastic bags in order to measure the morphological parameters (shoot length (cm), root length (cm), plant height, number of leaves per plant, leaf area (cm^2), and number of branches per plant) and for biomass assessment (fresh weight of root, fresh weight of shoot, fresh weight of total plant).

Chlorophyll Content Determination: The chlorophyll *a*, chlorophyll *b*, total chlorophyll content, and carotenoid content of the brinjal plant were estimated. Small sections of fresh leaves were cut and extracted with 80% acetone overnight. The extract was centrifuged at 14000rpm for 5 minutes. The absorbance of the supernatant was measured at 645nm, 663nm and 480nm using a spectrophotometer.

Statistical Analysis: The Pot experiment data was used to compute the treatment mean, standard error, and Duncan's Multiple Range Test (1960), were used to compute the treatment mean, standard error. This was done with the help of the IBM SPSS Statistics 20 software and the lab's computer resources [23].

RESULTS

No. of Leaves: The application of different concentrations of NaF and ZnOPs showed different significant changes in no. of leaves of *S. melongena* L. at 60 DAS. The maximum percentage increase in the no. of leaves was observed when plant was treated with NaF-1 (300ppm) and NaF-2 (400ppm) separately and in combination with five different concentrations of ZnOPs (100, 200, 300, 400, 500 ppm) as soil drench application was 92.4% and 15% at 60 DAS at the concentration of NaF-1+ZnOPs-5 (300ppm + 500ppm). Some variations were observed at 90 DAS; the maximum percentage increase was 38.2% at a concentration of NaF-1+ZnOPs-5. But on average, the gradual increase in the no. of leaves was observed with increasing the concentration of ZnOPs. The percentage decreases were observed at both NaF-1 (300ppm) and NaF-2 (400ppm) were -34.8% and -59.9% at 60 DAS, and at the same concentrations, -15.3% and -40.9% at 90 DAS. The higher no. of leaves was counted at NaF-1+ZnOPs-5, which is 25.6% for foliar application as compared to soil drench application (Figures 1A and 1B, Tables 1 and 2).



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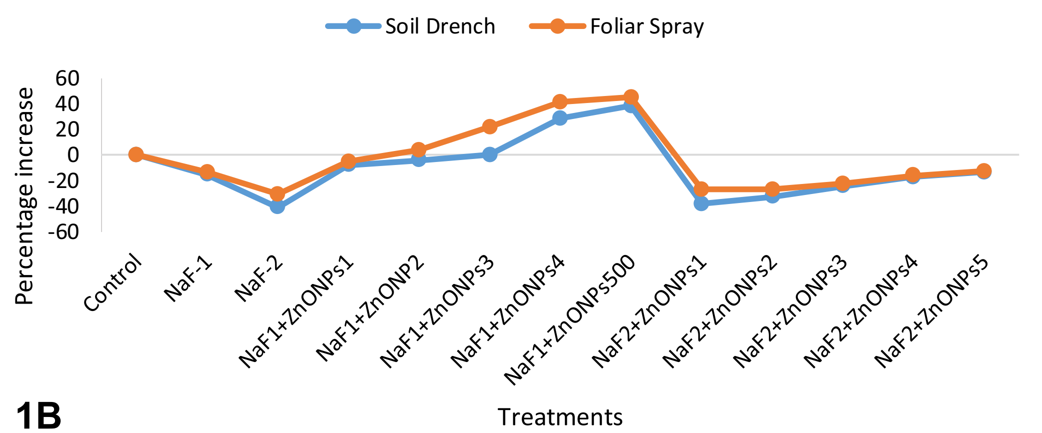

1B

Figures 1A and 1B. Trend showing reduction and increase in no. of leaves of brinjal treated with different NaF and ZnOPs concentrations. 1A: 60 DAS; 1B: 90 DAS.

Leaf Area: Plants applied with variable concentrations of ZnOPs and Na-F showed significant variations in the width and length of leaves. The maximum percentage increase was observed at 35.6% on NaF-1+ZnOPs-5 at 60 DAS and 93.3% at 90 DAS at the same concentration as soil drench. It indicated that this is a suitable concentration for plant growth. The plant showed the same increase in percentage when all these concentrations were applied as a foliar spray method, NaF-1+ZnOPs-5 showed the maximum increase in percentage at 133% at 90 DAS.

The percentage decrease was noted at NaF-2, -31.6% at 60 DAS and 12.6% at 90 DAS at same concentration. When leaf area was calculated for all the plants, different average values were observed that showed the same sequence for maximum

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percentage increase and percentage decrease for plants that were treated with two different methods (Figures 2A and 2B, Tables 1 and 2).

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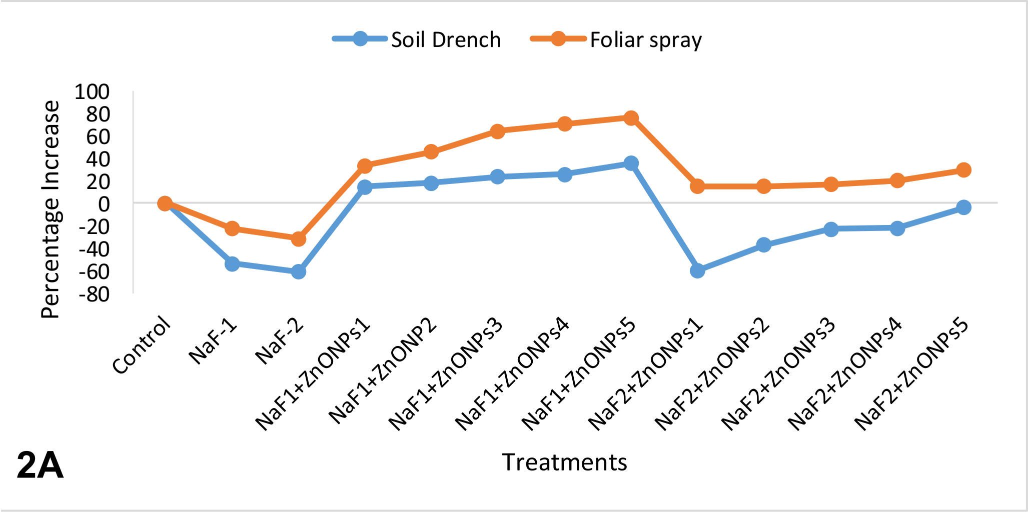


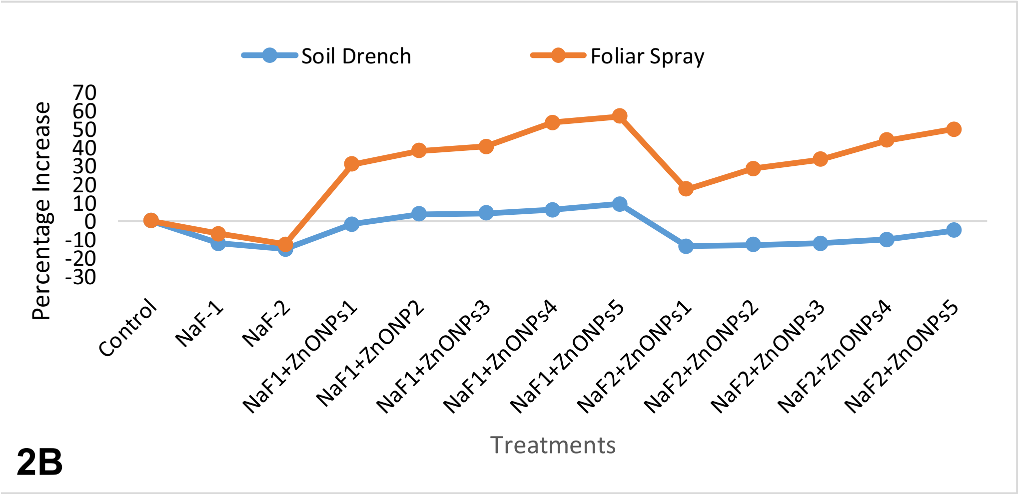
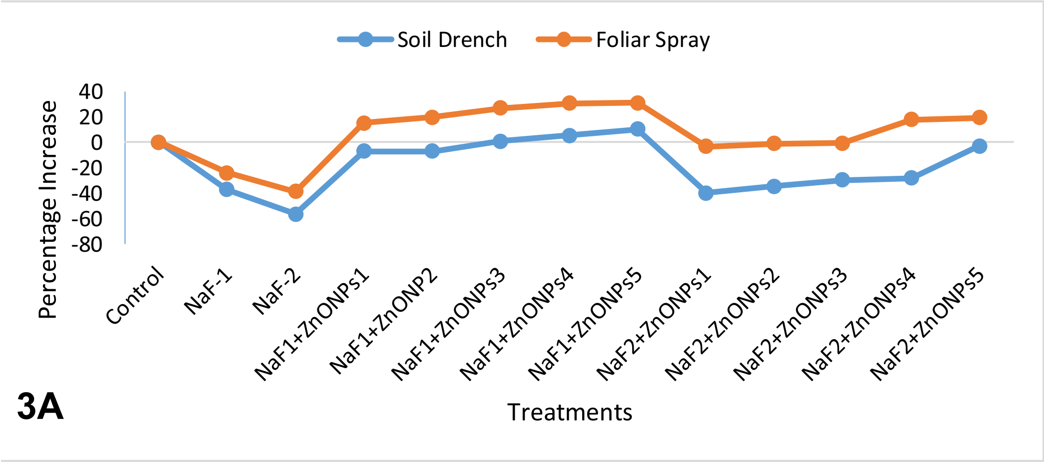


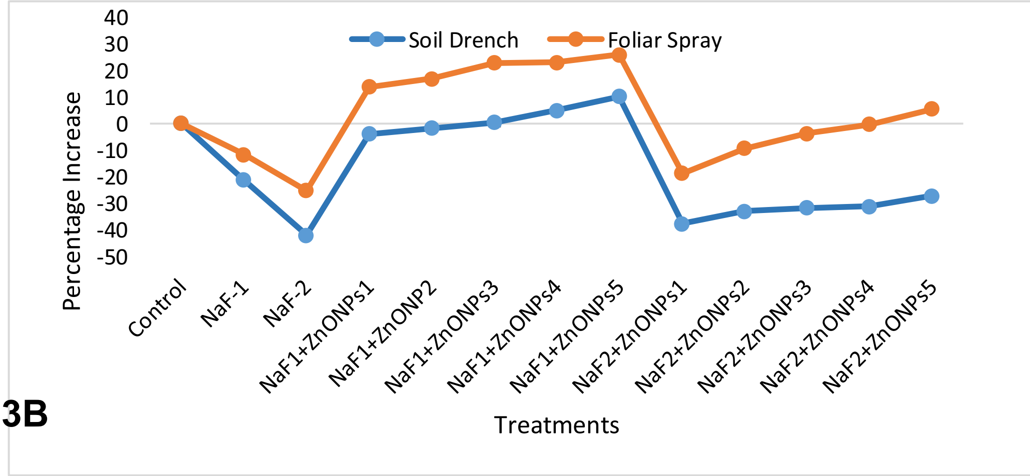
Figure 2A and 2B. Trend showing visible reduction and increase in leaf Area of brinjal treated with different NaF and ZnOPs concentrations. 2A: 60 DAS; 2B: 90 DAS.

Shoot Length: Shoot length is a major indicator of plant growth. As NaF and Zn nanoparticles were both applied together and NaF separately, they both showed significant growth changes during the whole experiment. The shoot length of *solanum melongena L.* when applied as soil drench showed considerable changes in length at 60 DAS which are 34.9cm for control, 21.9cm for NaF-1,15.1cm, 32.3cm for NaF-2, 32.3cm for NaF-1+ZnONPs-1, 35.1cm for NaF-1+ZnONPs-2, 36.7cm for NaF-1+ZnONPs-3, 38.5cm for NaF-1+ZnONPs-4, 20.9cm for NaF-1+ZnONPs-5, 22.7cm for NaF-2+ZnONPs-1, 24.4cm for NaF-2+ZnONPs-2, 25cm for NaF-

2+ZnONPs-3 and 33.8cm for NaF-3+ZnONPs-4. So, it has been shown that the higher shoot length expressed at NaF-1+ZnONPs-5 and the minimum shoot length at NaF-2. At 90 DAS, the shoot length showed the same changes as that of 60 DAS. The maximum increase in shoot length is at NaF-1+ZnONPs-5, which is 71.3 cm, and the minimum is at NaF-2, which is 37.5 cm.

Brinjal was also observed at 60 DAS and 90 DAS when all the applications were applied as foliar spray. The trend is the same. The greater the concentration of Zn nanoparticles, the greater the percentage increase. The lowest percentage increase was 18.5% at NaF-2 at 60 DAS and 46% at 90 DAS at the same concentration. The percentage increase gradually becomes higher as the concentration of ZnONPs increases. The maximum percentage increase is at NaF-1+ZnOPs-5, which is 31% at 60 DAS and 25.8% at 90 DAS at the same concentration. The percentage increase in shoot length is predicted to be greatest at the highest Zn nanoparticle concentration (Figures 3A and 3B, Tables 1 and 2).





Figures 3A and 3B. Trend showing visible reduction and increase in shoot length of brinjal treated with different NaF and ZnONPs concentrations. 3A: 60 DAS; 3B: 90 DAS.

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Root Length: The effects of different concentrations of ZnOPs (100, 200, 300, 400, 500 ppm) and two concentrations of NaF and their interaction with root length (*S. melongena L.)* during both stages were recorded. It was observed that NaF concentration causes a reduction in root length and ZnO-NPs interaction stimulates the increase in root length. The greater reduction in root length was observed at NaF of 7.73 cm and the maximum root length was observed at NaF-1 + ZnO-NPs-5 when plants were applied by the soil drench method with five different concentrations of ZnOPs in combination with 2 concentrations of NaF. The same trend was observed at the 2nd stage (90 DAS). The minimum growth of the root was 33.4 cm at NaF-2 and the maximum increase in root length was 53.7 cm at NaF-1+ZnONPs4 and NaF-1+ZnONPs-5. when applied with the same concentration as in stage 1 (60 DAS). In short, as the minimum growth at both stages is the same at the same concentration, it is expressed that there is a gradual increase in root length with an increase in ZnO NP concentration. So it can be suggested from the observation that NaF has a negative effect and ZnOPs have a positive effect on plant growth.

The same trend is expressed when the method of foliar spray is applied. The minimum growth is at NaF-2, which is 8.33cm, but the maximum is at NaF-2+ZnONPs-5, which is 28.3cm. It can be said that as the foliar spray amount of salt and nanoparticles applied is less, NaF-1 has less effect, and it showed maximum growth at NaF-1+ZnONPs-5. As first (60 DAS), the 2nd stage (90 DAS) has the maximum increase in growth at NaF-1+ZnONPs-5, which is 74.2cm (Figures 4A and 4B, Tables 1 and 2).

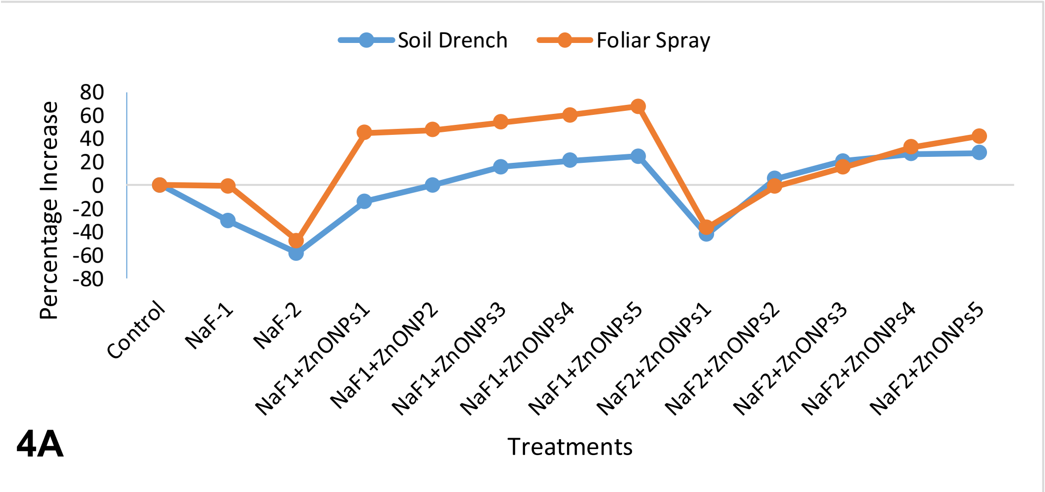

Figure 4A. Trend showing visible reduction and increase in root length of brinjal treated with different NaF and ZnO NPs concentrations. 4A: 60 DAS.

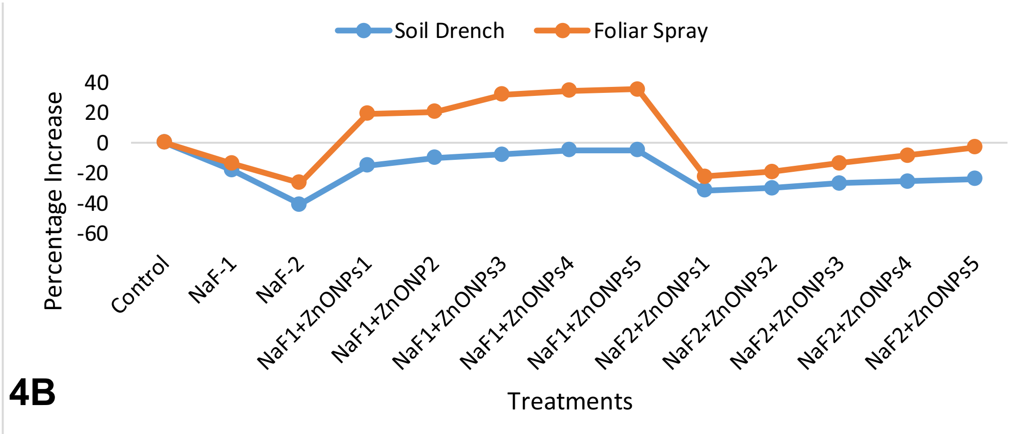

Figure 4B. Trend showing visible reduction and increase in root length of brinjal treated with different NaF and ZnO NPs concentrations. 4B: 90 DAS.

No. of Branches: The round purple variety of brinjal (*Solanum melongena* L.) that were selected to carried out the experimental work throughout the growing season (60 DAS and 90 DAS) to fine the changes in no. of branches per plant. Application of NaF and ZnOPs by both method (soil drench and foliar spray) cause variations in no. of branches at both stages (vegetative stage and at reproductive stage). When the plants were applied as soil drench the maximum no. of branches were observed at NaF-1+ZnOPs-5 which was 3 and minimum at NaF-2+ZnOPs1 at 60 DAS and the same trend was observed at 90 DAS, the maximum no. of branches were 13 at NaF-1+ZnOPs-5 and minimum 3.66at NaF-1 and NaF-2.

Both the stages were also observed when the plants were applied with all these concentrations as foliar spray. At 60 DAS, the maximum no. of branches and minimum no. of branches were same as that of soil drench application which were 3 and 0.66. At 90 DAS. The no. of branches maximum at NaF-1+ZnOPs-5 which was 15.3 and minimum at NaF-2+ZnONPs-1which is 3.66. So that the maximum no. of branches was observed at foliar application as compare to soil drench application (Figures 5A and 5B, Tables 1 and 2).

Biochemical Attributes: The impact of two concentrations of NaF (300ppm,400ppm) and five concentrations of ZnOPs (100, 200,300,400,500 ppm) on the pigments of brinjal at vegetative stage were observed. The chlorophyll *a*, chlorophyll *b* and total chlorophyll content were observed. The concentrations of NaF cause the pigments to decrease but with increasing the concentrations of nanoparticles will increase the pigment content. The minimum chlorophyll *a* was observed at NaF2 (400ppm) which was 1.39 mg/g fr. wt and minimum chlorophyll *b* was 0.43 mg/g fr. wt. at same concentration. The total chlorophyll content also low at NaF2 which was 1.82 mg/g fr. wt. at same concentration when applied with soil drench method and maximum at NaF1+ZnOPs 5 for chlorophyll *a* 1.57 mg/g fr. wt. for chlorophyll *b* 0.57mg/g fr. wt. and for total chlorophyll content was 2.14 mg/g fr. wt. over the control.

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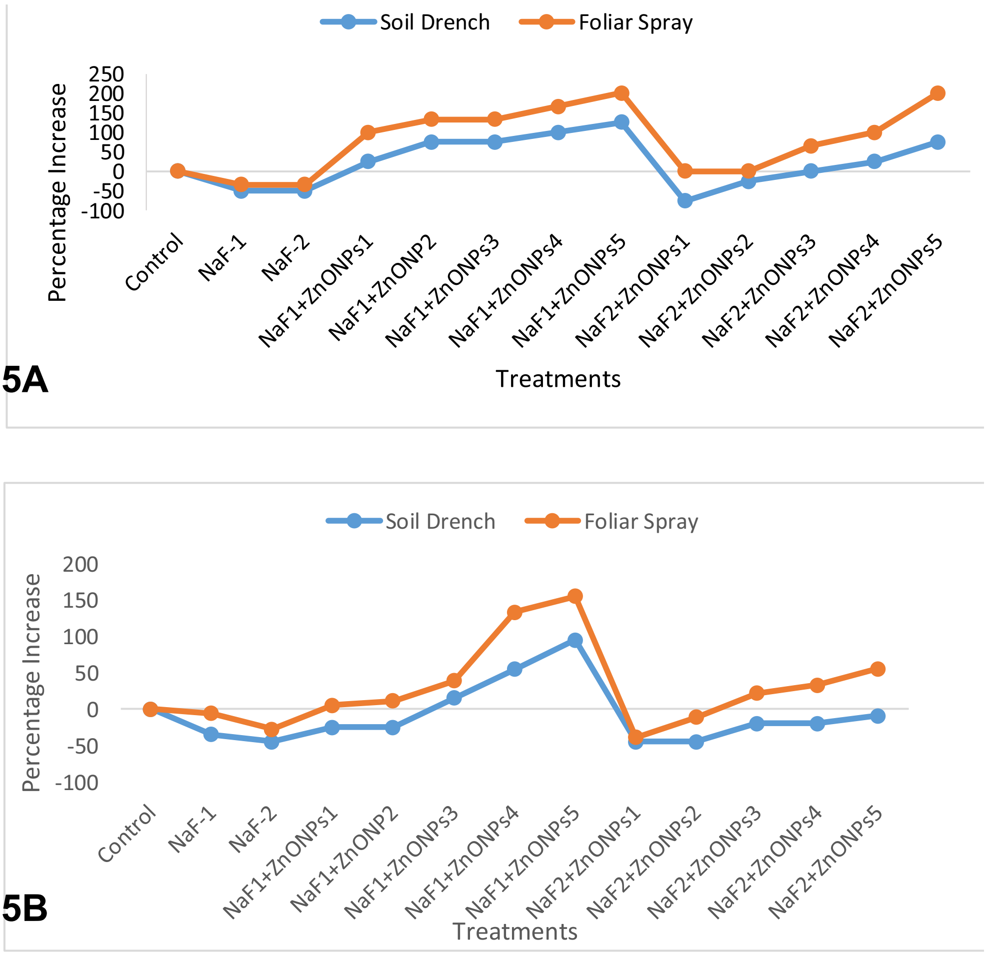

Figures 5A and 5B: Trend showing visible reduction and increase in no.of branches of brinjal treated with different NaF and ZnONPs concentrations. 5A: 60 DAS; 5B: 90 DAS.

At NaF2 chlorophyll *a* was 1.49, chlorophyll *b* was 0.43 and total chlorophyll content was 1.93 mg/g fr. wt minimum when applied as foliar spray. The maximum chlorophyll concentration was at NaF1+ZnOPs5 for chlorophyll a was 1.59 mg/g fr. wt, for chlorophyll b was 0.55 mg/g fr. wt and for total chlorophyll content was 2.14 mg/g fr. wt. The reduction in carotenoids is maximum at NaF-2 (400ppm) is 0.45 as soil drench application as compare to foliar spray. This effect decrease gradually with increasing the applied concentration of ZnOPs. The maximum growth was observed at NaF-1+ZnOPs-5 is 0.75 as foliar spray because of less effect of NaF on plant when applied as foliar spray as compare to soil drench (Table 3).

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Table 1: Yield Parameters of Round purple variety of *S. melongena* L. by soil drench method and foliar spray application method at 60DAS

Mode of Treatment	Treatments (ppm)	No.of Leaves	Leaf Area (cm2)	No.of branches	Shoot length (cm)	Root Length (cm)	Plant Height (cm)
Soil Drench	Control	13.3cd ±1.9	83.3e ±0.89	1.33ab ±0.88	34.9bc ±0.76	18.5cd ±0.70	53.4cd ±1.27
	NaF-1	8.66efg ±1.20	38i ±1.03	0.66ab ±0.33	21.9ef ±1.06	12.8e ±0.43	34.8h ±0.63
	NaF-2	5.33g ±0.88	19.4j ±0.53	0.66ab ±0.33	15.1g ±0.40	7.73f ±0.14	22.8i ±0.35
	NaF1+ZnONPs1	19b ±1.15	95.5d ±0.80	1.66ab ±0.33	32.3c ±1.20	15.9d ±0.52	48.3ef ±1.50
	NaF1+ZnONP2	22ab ±1.00	98.4c ±0.76	2.33ab ±0.66	32.3c ±0.66	18.5cd ±0.74	50.8de ±1.27
	NaF1+ZnONPs3	24.6a ±1.20	103b ±1.15	2.33ab ±0.88	35.1bc ±0.64	21.4ab ±1.44	56.6bc ±0.85
	NaF1+ZnONPs4	25.3a ±0.88	104b ±0.88	2.66ab ±0.88	36.7ab ±1.6	22.4a ±0.89	59.1ab ±0.85
	NaF1+ZnONPs5	25.6a ±0.88	113a ±1.20	03a ±1.15	38.5a ±1.18	23.1a ±0.95	61.6a ±0.55
	NaF2+ZnONPs1	06fg ±1.00	33.6j ±0.63	0.33ab ±0.33	20.9f ±0.20	10.7e ±0.68	31.6h ±0.66
	NaF2+ZnONPs2	08efg ±1.15	52.3h ±1.18	01ab ±0.57	22.7def ±0.46	19.5bc ±1.36	42.4g ±0.99
	NaF2+ZnONPs3	9.33ef ±1.45	64.1g ±1.02	1.33ab ±0.88	24.4de ±1.13	22.3a ±1.17	46.7f ±1.9
	NaF2+ZnONPs4	10.3de ±0.33	64.7g ±0.84	1.66ab ±0.88	25.0d ±0.58	23.5a ±0.32	48.5ef ±0.89
	NaF2+ZnONPs5	15.3c ±1.20	80.3f ±0.68	2.33ab ±0.33	33.8c ±0.38	23.6a ±0.87	57.4b ±1.01
Foliar Spray	Control	12.6ef ±0.33	75.6i ±0.92	01a ±0.47	30.3d ±0.66	16.9e ±0.52	46.9d ±0.40
	NaF-1	10.6fg ±0.88	58.4j ±0.46	0.66a ±0.33	23e ±0.64	16.8e ±1.16	39.8e ±0.52
	NaF-2	9.33g ±0.88	51.7k ±0.40	0.66a ±0.33	18.5f ±0.86	8.83f ±1.42	27.3f ±0.88
	NaF1+ZnONPs1	22.6c ±1.20	101e ±0.88	02a ±0.57	34.9c ±0.35	24.6bc ±0.88	59.6c ±0.57
	NaF1+ZnONP2	24c ±1.73	110d ±1.45	2.33a ±0.66	36.2bc ±1.10	24.9abc ±1.34	61.1c ±1.51
	NaF1+ZnONPs3	27.3b ±0.88	124c ±0.88	2.33a ±0.66	38.4ab ±0.86	26ab ±0.57	64.4b ±1.27
	NaF1+ZnONPs4	30.6a ±1.45	129b ±1.45	2.66a ±0.88	39.6a ±0.66	27.2ab ±1.04	66.8ab ±1.18
	NaF1+ZnONPs5	31.5a ±0.78	133a ±0.88	03a ±1.7	39.7a ±0.93	28.3a ±1.20	68a ±0.96
	NaF2+ZnONPs1	10.6fg ±0.88	86h ±0.43	01a ±0.57	29.3d ±1.74	10.7f ±1.15	40e ±0.60
	NaF2+ZnONPs2	12fg ±0.57	87.1h ±0.32	01a ±1.73	29.9d ±0.37	16.7e ±0.67	46.6d ±0.33
	NaF2+ZnONPs3	13.3ef ±0.66	88.2h ±0.51	1.66a ±0.88	30d ±1.48	19.5de ±1.04	49.5d ±0.86
	NaF2+ZnONPs4	15.3e ±0.66	91g ±0.63	02a ±1.15	35.7bc ±0.39	22.4cd ±0.29	58.2c ±0.23
	NaF2+ZnONPs5	18.6d ±0.88	98.0f ±0.47	03a ±1.15	36.1bc ±1.08	24bcs ±1.86	60.2c ±1.67

Each treatment mean is sum of three replicates and ± represents standard error (SE). Within each parameter values not followed by same letter are significantly different at Duncan's multiple range test. NaF: Sodium fluoride; ZnONPs: Zinc Nanoparticle;

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Table 2: Yield Parameters of Round purple variety of *S. melongena* L. by soil drench method and foliar spray application method at 90DAS.

Mode of Treatment	Treatments (ppm)	No.of Leaves	Leaf Area (cm^2)	No.of branches	Shoot length (cm)	Root length (cm)	Plant Height (cm)
Soil Drench	Control	36.6b ±0.88	85.5c ±0.61	6.66cd ±1.45	64.7cd ±0.50	56.5a ±0.61	121b ±0.88
	NaF-1	31cde ±1.52	75ef ±0.63	4.33d ±0.88	50.8e ±0.76	44.5e ±0.85	94.6e ±1.45
	NaF-2	21.6g ±0.88	72.5f ±0.78	3.66d ±0.66	37.5h ±0.95	33.4i ±0.99	71j ±1.15
	NaF1+ZnONPs1	33.6bcd ±0.66	84.0c ±1.13	05cd ±0.57	62.1d ±0.73	47.8d ±1.07	110d ±0.57
	NaF1+ZnONP2	35bc ±1.52	88.7b ±1.00	05cd ±0.57	63.5cd ±0.45	50.7c ±1.17	114c ±1.00
	NaF1+ZnONPs3	36.6b ±1.20	89.1b ±1.01	7.66bc ±0.33	64.9c ±0.20	52.0bc ±1.17	117c ±0.75
	NaF1+ZnONPs4	47a ±1.0	90.7ab ±0.49	10.33ab ±0.66	67.9b ±1.39	53.7b ±0.14	122b ±1.45
	NaF1+ZnONPs5	50.6a ±1.20	93.3a ±0.69	13a ±1.15	71.3a ±0.86	53.7b ±1.37	125a ±1.00
	NaF2+ZnONPs1	22.6g ±1.66	73.8ef ±0.77	3.66d ±0.66	42.5g ±1.16	38.6h ±0.71	81.1i ±0.98
	NaF2+ZnONPs2	24.6fg ±1.20	74.5ef ±0.54	3.66d ±1.20	43.3g ±0.56	39.5gh ±0.55	82.8hi ±0.92
	NaF2+ZnONPs3	27.6ef ±1.33	75ef ±12.2	5.33cd ±0.88	44.1g ±0.79	41.2fg ±0.69	85.3gh ±0.918
	NaF2+ZnONPs4	30.3de ±1.2	76.8e ±1.4	5.33cd ±1.20	44.5g ±0.96	42.0f ±0.08	86.6g ±0.91
	NaF2+ZnONPs5	31.6cde ±0.88	81.2d ±1.53	06cd ±1.00	47.0f ±0.88	43.4ef ±0.53	90.4f ±1.34
Foliar Spray	Control	37cd ±1.00	84.8h ±0.95	06cdefg ±0	61.6d ±0.20	54.7c ±0.85	116c ±1.00
	NaF-1	32ef ±1.15	79i ±1.37	5.66defg ±0.88	54.3e ±0.53	47.1e ±0.61	101f ±1.07
	NaF-2	25.6h ±1.2	74.1j ±1.26	4.33g ±0.88	46.0g ±1.22	40.2g ±0.77	86.7h ±1.74
	NaF1+ZnONPs1	35de ±1.3	111ef ±0.88	6.33cdef ±0.33	70.1b ±1.05	65.2b ±1.10	135b ±1.00
	NaF1+ZnONP2	38.3c ±1.0	117d ±0.88	6.66cdef ±0.66	72.0b ±1.29	65.9b ±1.48	138b ±1.15
	NaF1+ZnONPs3	45b ±1.5	119d ±0.66	8.33bc ±0.33	75.7a ±0.54	72.1a ±1.07	147a ±1.46
	NaF1+ZnONPs4	52.3a ±0.6	130b ±0.57	14a ±1.00	75.8a ±0.68	71.5a ±0.46	149a ±1.19
	NaF1+ZnONPs5	53.6a ±0.8	133a ±0.30	15.3a ±1.66	77.5a ±0.26	74.2a ±0.70	152a ±0.88
	NaF2+ZnONPs1	27h ±1.0	99.4g ±1.02	3.66fg ±0.33	50f ±0.81	42.3fg ±0.32	92.3g ±0.89
	NaF2+ZnONPs2	27h ±0.7	109f ±0.66	5.33dfg ±0.88	55.8e ±0.50	44.1f ±1.12	100f ±1.50
	NaF2+ZnONPs3	28.6gh ±1.2	113e ±0.88	7.33bcde ±0.33	59.2d ±0.88	47.2e ±0.48	106e ±0.93
	NaF2+ZnONPs4	31fg ±1.0	122c ±0.57	08bcd ±1.00	61.4d ±1.28	50.0d ±0.29	111d ±1.44
	NaF2+ZnONPs5	32ef ±0.3	127b ±0.86	9.33b ±0.33	64.9c ±0.70	53.1c ±0.73	118c ±1.42

Each treatment mean is sum of three replicates and ± represents standard error (SE). Within each parameter values not followed by same letter are significantly different at Duncan's multiple range test. NaF: Sodium fluoride; ZnONPs: Zinc Nanoparticle;

[Now published in full after the initial publication as an Epub ahead of print August 8, 2022 at www.fluorideresearch.online/epub/files/200.pdf]

Table 3: Estimation of Chlorophyll *a* and Chlorophyll *b* and total chlorophyll content in brinjal leaves as soil drench and foliar spray at 60DAS.

Mode of Treatment	Treatments (ppm)	Chlo. a (mg/g fr wt.)	Chlo b (mg/g fr wt.)	Carotenoid (mg/g fr. wt.)	Total Chlo (mg/g fr. wt.)
Soil Drench	Control	1.6 ±0.006	0.51 ±0.015	0.69 ±0.006	2.12 ±0
	NaF-1	1.46 ±0.013	0.47 ±0.007	0.52 ±0.006	1.93 ±0.016
	NaF-2	1.39 ±0.015	0.43 ±0.007	0.45 ±0.01	1.82 ±0.012
	NaF1+ZnONPs1	1.48 ±0.008	0.53 ±0.015	0.53 ±0.003	2.01 ±0.003
	NaF1+ZnONP2	1.53 ±0.008	0.54 ±0.007	0.58 ±0.003	2.08 ±0.012
	NaF1+ZnONPs3	1.56 ±0.006	0.58 ±0.007	0.64 ±0.01	2.14 ±0.008
	NaF1+ZnONPs4	1.61 ±0.008	0.62 ±0.007	0.71 ±0.02	2.24 ±0.008
	NaF1+ZnONPs5	1.65 ±0.011	0.7 ±0.015	0.73 ±0.01	2.34 ±0.013
	NaF2+ZnONPs1	1.39 ±0.013	0.45 ±0.007	0.46 ±0.01	1.85 ±0.015
	NaF2+ZnONPs2	1.43 ±0.003	0.45 ±0.007	0.48 ±0.006	1.89 ±0
	NaF2+ZnONPs3	1.46 ±0.003	0.48 ±0.007	0.51 ±0	1.94 ±0.003
	NaF2+ZnONPs4	1.52 ±0.003	0.53 ±0.007	0.53 ±0.01	2.05 ±0.003
	NaF2+ZnONPs5	1.57 ±0.011	0.57 ±0.027	0.56 ±0.01	2.14 ±0.02
Foliar Spray	Control	1.57 ±0.027	0.5 ±0.015	0.66 ±0.006	2.07 ±0.013
	NaF-1	1.54 ±0.007	0.45 ±0.015	0.56 ±0.006	2 ±0.01
	NaF-2	1.49 ±0.020	0.43 ±0.007	0.49 ±0.008	1.93 ±0.02
	NaF1+ZnONPs1	1.52 ±0.015	0.48 ±0.007	0.56 ±0.006	2.01 ±0.01
	NaF1+ZnONP2	1.53 ±0.007	0.5 ±0.015	0.61 ±0.003	2.04 ±0.01
	NaF1+ZnONPs3	1.56 ±0.015	0.53 ±0.013	0.65 ±0.016	2.09 ±0.012
	NaF1+ZnONPs4	1.62 ±0.007	0.58 ±0.013	0.71 ±0.006	2.21 ±0.005
	NaF1+ZnONPs5	1.68 ±0.015	0.64 ±0.015	0.75 ±0.006	2.32 ±0.013
	NaF2+ZnONPs1	1.5 ±0.015	0.45 ±0.015	0.5 ±0.01	1.95 ±0.006
	NaF2+ZnONPs2	1.51 ±0.007	0.46 ±0.02	0.52 ±0.006	1.98 ±0.011
	NaF2+ZnONPs3	1.53 ±0.013	0.48 ±0.007	0.55 ±0.006	2.01 ±0.006
	NaF2+ZnONPs4	1.56 ±0.015	0.52 ±0.015	0.56 ±0.006	2.09 ±0.011
	NaF2+ZnONPs5	1.59 ±0.007	0.55 ±0.007	0.61 ±0.006	2.14 ±0.003

Each treatment mean is sum of three replicates and ± represents standard error (SE). Within each parameter values not followed by same letter are significantly different at Duncan's multiple range test. NaF: Sodium fluoride; ZnONPs: Zinc Nanoparticle;

DISCUSSION

Salinity is an abiotic stress that reduces plant growth, quality, productivity and development. Salt stress affects nearly 2.1 % of total dry weight and 19.5 %of irrigated land, with these figures steadily rising [24]. Major agricultural methods may become unsustainable as a result of climate change and a rising human population [25]. Industrial pollution, incorrect pesticide use, and an improper irrigation system could make salinization worse at the global level [26]. Fluorine is the 13th most plentiful element in the universe, with around 950 mg/L stored in fluoride form in the earth's crust [27] and become a hazardous element in the food chain. Plant output declines as a result of salt stress. Salt stress has negative effects on plants at all stages of development and can lead to crop loss [28].

Safflower is given a fluoride stress treatment, and it can tolerate up to 200 mg of fluoride/kg of soil. Because of fluoride's toxicity and an increase in oxidative stress in the roots and leaves, fluoride levels can lead to further reductions in safflower growth [29]. Reduced pH, fresh weight, and relative water content of pineapple mint are caused by increased saline stress. The deleterious impact of salinity, which eventually reduce plant yield, are most frequently responsible for growth inhibition and biomass reduction [30].

The shoot and root length, number of leaves along with leaf area showed dose-related reductions. The NaF caused more toxicity when applied as soil drench method than that of foliar application [31]. A previous study has shown that NaF reduces seedlings growth when toxicity of NaF enhanced. NaF2 (400ppm) reduced shoot and root length, number of leaves along with leaf area, and biomass more than NaF1 (300ppm) and was controlled by soil drenching rather than foliar spray.

The application of nanoparticles (NPs) in agriculture is primarily responsible for improving the physical and chemical features of soil, particularly water holding capacity, in order to manage fertilizer distribution [32]. Zinc oxide NPs is a metal nanoparticle, has amazing antibacterial, physical, and optical properties, as well as a significant potential for agricultural enhancement [33]. Nanoparticles can be made through physical, chemical, or biological processes [34]. Green synthesis is a prominent approach in nanotechnology. Medicinal effects are unique characteristics of tropical plants that have metallic nanoparticles associated with them. Due to their small size, physical properties, and orientation, nanoparticles are widely employed and have the potential to influence the performance of materials with which they come into contact. Neem (*Azadirachta indica*) leaf extracts aqueous solution and zinc nitrate solution were used for green synthesis [35]. During study, the ZnOPs were synthesized using $ZnSO_4$ solution and an equal volume of *syzigium cumuni* L. leaf powder extract. The mixture is next tested for four hours for the formation of nanoparticles.

The higher concentrations of ZnOPs revealed negative impacts on eggplant growing in tissue culture and reduced the growth of seedling. Nevertheless, ZnOPs boosted eggplant growth in the field. Plant height, number of leaves, and petiole length were 17.24cm 46.67, and 9.22cm respectively [36]. Adrees et al., reported that foliar application of ZnOPs enhanced the growth, biomass and chlorophyll content of wheat plants [37]. The exogenous application of ZnOPs cause Zn accumulation in wheat plant that are efficient source of Zn in growing seedlings and enhanced metabolic activities in wheat plant [38]. The ZnOPs absorption is mainly through the leaf cuticle which was observed in sunflower and wheat[39].

In order to investigate the process of absorption and attachment, ZnOPs (30 nm) labeled with FITC (fluorescein isothiocyanate) were applied foliar to wheat leaf. Laser confocal microscopy was used to reveal the pathway. The result showed that the main passage for ZnOPs is stomata to cross epidermis of leaf. Then accumulation of nanoparticles causes the Zn ion to release in apoplasts following the absorption by mesophyll cells of leaf. [40]. The foliar application of nanoparticles used less quantity and might be less economical approach as compare to soil amendment. Whereas, the ZnOPs as soil application increased the Zn content which cause enhanced growth of wheat plant [41].

Five different concentrations of ZnOPs were administered to the NaF-stressed brinjal plants. The ZnOPs treatments boost plant growth and development. Higher concentration of ZnOPs (500ppm) reduced NaF stress that leads to increased plant growth. In comparison to soil drench, the effect of ZnOPs as foliar spray on these stressed plants is greater, as NaF1 (300ppm) has less influence on brinjal plants than NaF2 (300ppm) as a soil drench.

CONCLUSIONS

Sodium fluoride has negative impacts on plant growth and metabolic processes, which negatively affected seed germination parameters and further reduced the plants' growth and development. Results of current study advocate that when NaF is applied at 400ppm as a soil drench method it causes more reduction in plant growth than when applied at 300ppm. Foliar applied zinc oxide nanoparticles (ZnOPs) enhanced plant biomass and capped the worst effect of NaF on brinjal plants by triggering its physio-biochemical processes through maintaining chlorophyll *a* and *b* contents. Furthermore, ZnOPs improved the carotenoid content and enhance plant height under NaF stress. ZnOPs(500ppm) was more effective in reducing the negative effect of NaF at both growth stages then lesser concentrations of ZnOPs (100ppm, 200ppm, 300ppm and 400ppm). However, additional comprehensive research work under field conditions seems mandatory to reconnoiter the mechanism of ZnOPs for NaF stress mitigation and growth improvements of F-stressed brinjal plants.

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