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Keywords: *sunflower, nano ZnO, nano ZnS, NPs, FE-SEM, XRD, boron, $ZnSO_4$, foliar spray.*

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FOLIARAPPLICATIONOFGREENSYNTHESIZEDZINCSULPHIDEANDZINCOXIDENANOPARTICLESENHANCESGROWTHROOTATTRIBUTESYIELDANDOILQUALITYOFSUNFLOWERHELIANTHUSANNUUSL

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Keywords: sunflower, nano ZnO, nano ZnS, NPs, FE-SEM, XRD, boron, $ZnSO_4$, foliar spray.

I. Introduction

Oilseed crops constitute a major part of nutrition in human beings and globally, sunflower (*Helianthus annuus* L.) ranks second next to soybean among annual field crops grown for edible oil. Micronutrient deficiency is a main constraint in oilseed production which affects the growth, yield and oil quality. Among micronutrients zinc deficiency is prominent in Indian soils and if oilseed crops like sunflower is cultivated in nutrient deficient soils the growth, yield and quality parameter of the crop is severely impaired. Similarly, sulphur is important as secondary nutrient for protein synthesis and is known to be a constituent of sulphur containing amino acids like cystine, cysteine and methionine which improves oil quality. Sulphur application on sulphur deficient soils can augment the supply of edible oils considerably [(1)]. Boron is another essential microelement for sunflower, helps in several processes like flowering, pollen germination, fruiting processes and seed setting. Hence, supply of balanced nutrition with enhanced nutrient use efficiency can help to achieve full yield potential of crop.

The desire to improve efficient use of zinc arises because zinc fertilizers are not always adequate to overcome the crop production constraints. Zinc fertilizers rapidly form insoluble complexes in the soil, rendering them unavailable for the plant uptake. Many efforts are being done in the field of nanotechnology to deal with various metals and their oxides as a source of essential plant nutrients [(2, 3)]. The physico-chemical and biological advantages of nano scale ZnS and ZnO has been thoroughly reviewed[(4)]. The use of ZnO NPs has a potential advantage, though the potential adverse effects are reported[(5,6)]. There are reports indicating that nano scale zinc oxide particles increased stem and root growth and pod yield of groundnut when compared to zinc sulphate application [(7)]; fruit yield of tomato [(8)] and nano iron oxide particles improved soybean yield [(9).]

Foliar application of trace elements being a common practice among farming community, the use of nanoparticles as foliar spray will be advantageous as it would reduce the bulk besides improving the efficiency. Use of a high analysis source of zinc such as $ZnSO_4$ is not soluble in water and foliar application of $ZnSO_4$ may not penetrate the leaf tissue effectively due to their bigger size. In such situations sources of zinc such as metal oxides if used as nano particles and in less than

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100 nm dimensions can considerably modify their physico-chemical properties compared to bulk [10]. Hence an effort is needed for alternative optimum fertilization strategies for enhancing or maintaining the yield levels. Our previous study evaluated the foliar application of nano ZnS with different concentrations (100, 200, 300, 400 and 500 ppm) at 35 and 55 DAS. Foliar application of nano ZnS 400 ppm at 35 DAS registered significantly improved plant height from 9.5 to 17.6 % between 40 DAS and harvest, number of green leaves plant^{-1} (17.13-33.40 %), leaf area (14.35-61.32%), leaf area duration (38.9-56.8 %) over water spray and soil application of zinc sulphate [11].

The zinc nanoparticles possess high surface area, promote better absorption and translocation even at low concentrations have proved to be effective in enhancing plant growth, development and yield of oil seed crops. Similar results with the use of nano ZnS were observed in sunflower [12] and with nano ZnO in groundnut in the field [7]. Although many researchers have reported the effects of nanoparticles and conventional fertilizers in oilseed crops, the information on combination of these with time of foliar application during the growth stages of sunflower to assess the yield enhancement is meager. In view of the above facts the present study was carried out with the objective to know the effect of foliar application of nano zinc particles on growth, yield and oil quality of sunflower.

II. Material and Methods

a) Physico-chemical properties of the soil

A composite soil sample was collected and the soil was air dried and sieved through 2 mm mesh. The initial properties of soil were analyzed following standard protocols.

Table 1: Properties of soil used in the pot experiment

Texture	Course sand (%)	Fine sand (%)	Silt (%)	Clay (%)	pH	ECe (d sm^{-1})	Organic carbon (g kg^{-1})	Available N (kg ha^{-1})	Available P_2O_5 (kg ha^{-1})	Available K_2O (kg ha^{-1})	Available S (ppm)	Available Zinc (mg kg^{-1})
Clay loam	34.70	30.40	16.30	18.60	7.8	0.80	0.51	145.60	13.67	255.75	7.80	0.48

b) Green synthesis of ZnO and ZnS NPs

Colloidal stable Zn-chitosan NPs with a size range of 38-60 nm for ZnS and ZnO have been successfully synthesized through green synthesis in the presence of a biopolymer, chitosan. Solution of nano ZnO was prepared by dispersing the commercial grade nanopowder (Sigma-Aldrich, USA) in Milli-Q water through ultrasonication (250W, 50kHz) for 30 minutes using chetosan biopolymer as substrate. Similarly, nano zinc sulphide was synthesized in a one-step colloidal synthesis of bio-compatible water-soluble ZnS quantum dot/chitosan nanoconjugates [13].

c) Characterization of ZnO and ZnS NPs

The estimation of the dimension of the nanoparticles was based on band gap energy value of UV spectrophotometer, particle size analyzer and size of the ZnS nano-crystals was estimated using the empirical model published in the literature [14, 15]. The concentration of ZnS and ZnO in samples was determined by drying a known volume of solution in hot air oven at 60 ^{0}C for 6 hours. X-ray Diffraction analysis (XRD) and UV-Visible spectroscopy (SP-UV500VDB, Spectrum Instruments) were used to characterize these NPs.

d) Pot experiment

The achenes of sunflower cv. RSFH-130, a short duration hybrid (95–98 days) were used. Initially three healthy achenes were sown in each pot and one healthy plant was retained after 15 days. Each of the treatment had three pots as repetitions in a complete randomized block design. A total of 72 pots were prepared for destructive sampling at three growth stages (Plate 1).

Plate 1: A view of the pot experiment

Each pot was fertilized with the recommended dose of nitrogen, phosphorus and potassium at the rate of 35:50:35 kg N, P_2O_5 and K_2O per hectare in the form of urea, diammonium phosphate and muriate of potash. Farm yard manure was incorporated 15 days before sowing in all the pots @ 8 t ha^{-1}. Spray volume for each concentration determined by measured quantity of water utilized for spray on one plant based on calculated total required quantity of spray volume to whole plants for each concentration. The pots of each set of sampling were randomly rotated from time to time to avoid over exposure to environmental variations in the screen house,. The minimum and maximum temperature increased during the experiment reaching 22.6^{o}C and 39.3^{o}C respectively at late booting stage. The maximum temperature exceeded 30^{o}C one week before buttoning and 4-5 days before anthesis. However, temperature remained stable in the range 22-26^{o}c during most of crop cycle.

e) Analysis of below ground parameters

The root washing for harvest samples was performed using 0.5% solution of sodium hexameta phosphate and washed roots were placed in root scanner trays in the root scanner computer system (Regent- STD 1600 + which uses a win RHIZO™ 2013 software programme; Regent instrument, Canada). Parameters such as root length (cm), average diameter (mm), root volume (cm^3), root length volume ($cm\ m^{-3}$), root surface area (cm^2) and forks class were analyzed.

f) Harvesting and threshing

The crop was harvested when dorsal side of the capitulum turned to lemon yellow colour. The heads from the pots were cut and seed yield was recorded after thorough drying. The stalks were left in pots for a week and were cut at ground level with the help of sickle and weight was recorded. The plant samples were oven dried at 65°c.

Various biometric observations, plant height, number of green leaves, leaf area, leaf area index, total dry matter, seed weight, stalk yield, seed oil content and harvest index were recorded at final harvest.

g) Sulphur and Zn uptake

S and Zn uptake at harvest was computed using the formula:

Sulphur or Zn uptake (kg ha^{-1}) = [S or Zn (%) x above ground dry matter (kg ha^{-1})] / 100

Oil content and oil yield

Dried achenes of sunflower drawn from each treatment were used for estimation of per cent oil content using Nuclear Magnetic Resonance (NMR) Spectrophotometer facility at Indian Institute of Oil Seeds Research, Hyderabad, India. Oil yield was calculated by multiplying the oil per cent with seed yield as follows:

Seed oil yield (g plant^{-1}) = [Seed oil content (%) x Seed yield (g plant^{-1})]/100

h) Statistical analysis

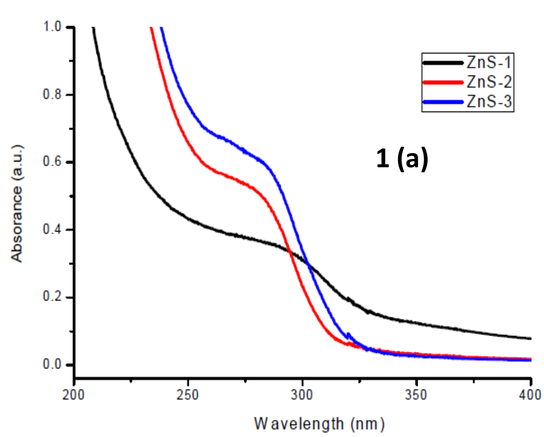
The data were analyzed statistically using the Fisher's analysis of variance. Least significant difference (LSD) test at 5% probability level was used to compare the differences among treatments' means when F-value is significant for observations [29].

III. Results and Discussion

a) Particle size analysis

The UV– visible absorption spectra of ZnS was obtained at different reaction time (Fig. 1-a) and the excitation wavelength was 275.6 nm (Fig. 2-a). The absorption peaks at 30 and 35 h exhibited great blue shift and strong intensity. Such a strong peak is known to arise due to quantum confinement effect, which occurs when the particle size becomes comparable with, or smaller than, the Bohr radius of excitation conformity as done by Vogel *et al.*[16].

A typical XRD pattern of green ZnO NPs was found in the range 5^0-50^0 (Fig. 1-b). The diffraction peak at 2θ with crystal planes corresponded in the order 33.62^0 (100), 35.2^0 (002), 39.30^0 (101), 48.68^0 (102), 58.26^0 (110), 75.26^0 (103), and 88.28^0 (200). Average size of ZnO green NPs was determined as 38 nm with crystalline structure from the width of dominant peaks (100) and (101) reflections according to the Debye-Scherrer's equation. The broadening of the peaks can be attributed to the decrease in the particle size of the synthesized ZnO. An absorption peak was observed in each spectrum at 383.8 nm as a characteristic band for the zinc oxide indicating the high purity of the synthesized ZnO NPs. The average size of green ZnS nanoparticles estimated from particle size analyzer was 60 nm. This is fairly in good agreement with the size estimated by Scherrer's equation (Fig. 2-b).



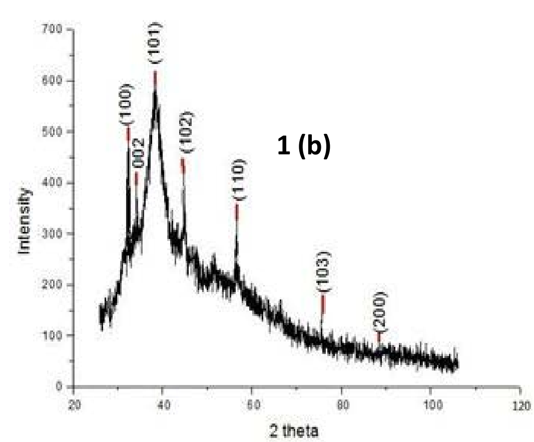


Fig. 1: (a). UV–vis spectroscopy analysis of green ZnS-chitosan conjugates synthesized at different pH 4.02, 5.0, and 6.0 of sample 1, 2, and 3 respectively. Based on E_{QD} results, the average sizes (diameter) were calculated were 4.7 ± 0.1, 4.4 ± 0.1 and 3.8 ± 0.1 nm for pH = 4.0, 5.0 and 6.0, respectively. Fig. 1(b). XRD patterns of the ZnO-chetosan conjugated NPs.

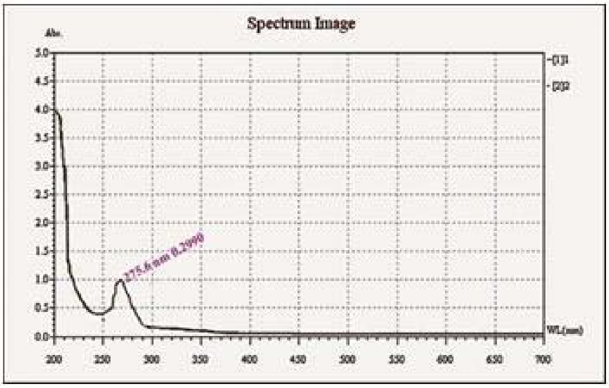


Fig. 2 (a): The UV– visible absorption spectra of ZnS

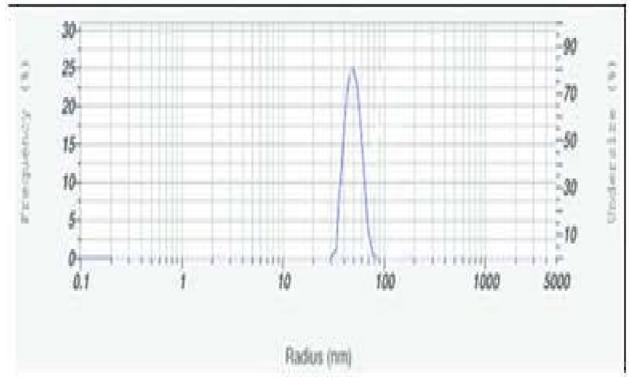


Fig.2 (b): Particle size analysis of ZnS

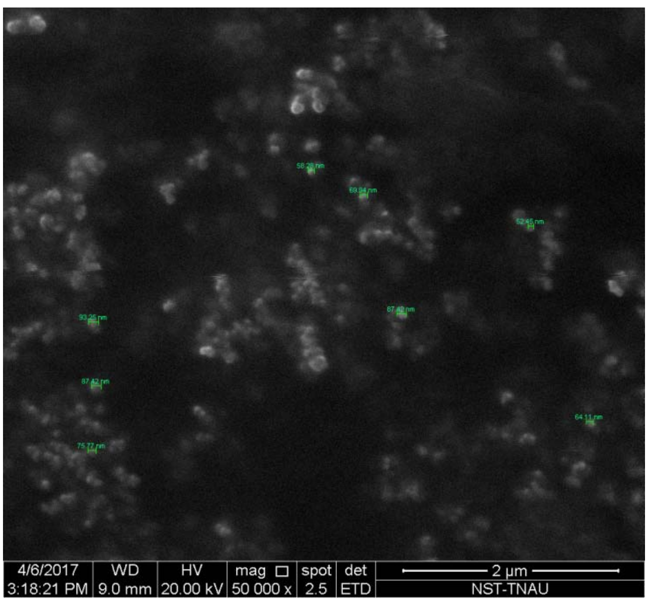


Plate 2 (a): FE-SEM images of sunflower leaf sprayed with nano ZnS @ 400 ppm

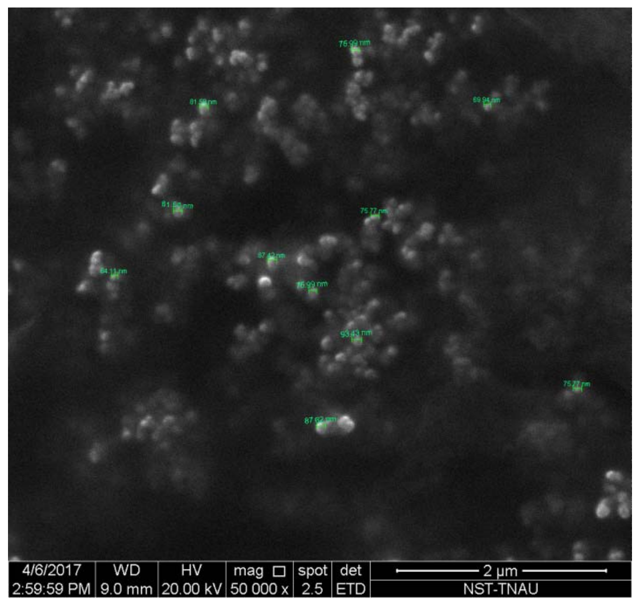


Plate 2(b): FE-SEM images of sunflower leaf sprayed with nano ZnO @ 1000 ppm

The FE-SEM images of sunflower leaf sampled after 24 hrs of spray with 400 ppm nano ZnS and 1000 ppm nano ZnO are shown in Fig 2 (a & b). The EDAX analysis of nano ZnS particles revealed the presence of zinc with weight per cent (0.47 %) and atomic per cent (0.10 %) and for 400 ppm nano ZnS also revealed the presence of zinc as weight per cent (0.46 %) and atom per cent (0.09 %).

The comparative absorption and deposition of zinc particles are depicted in Fig. 3 (a-d) indicated higher absorption of nano particles into stomatal aperture compared to conventional $ZnSO_4$. The EDX spectrum analysis revealed the presence of zinc element in leaf sample to an extent of 0.09 per cent by atomic weight. This clearly supported the fact that nano formulations were effective in reaching inner surface of leaf and enhanced the dry matter of photosynthetic surfaces. The bioaccumulation increased with application of ZnS nanoparticles when sprayed on foliage and effectively penetrated through stomatal pores. Similar results for ZnO nano particles were reported by Khanm *et al*. (2018) in tomato. Usually the size of stomatal pores generally ranges from few nanometres to 30 μm in different crops. Fricker and Willmer [17] opined that the formulation prepared at nano scale and sprayed on foliage could effectively be deposited on leaf surface and have made their entry into the leaf pores. Due to all these, nanoparticles are more efficient than conventional fertilizers.

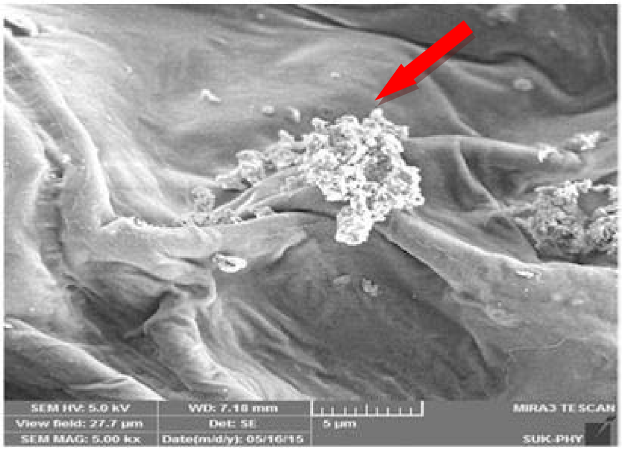


Plate 3 (a): FE-SEM image of reference standard leaf of sunflower sprayed with 5000 ppm $ZnSO_4$ at 35 days after sowing. Arrow shows the dense deposition of conventional $ZnSO_4$ on the leaf surface (x=5μ).

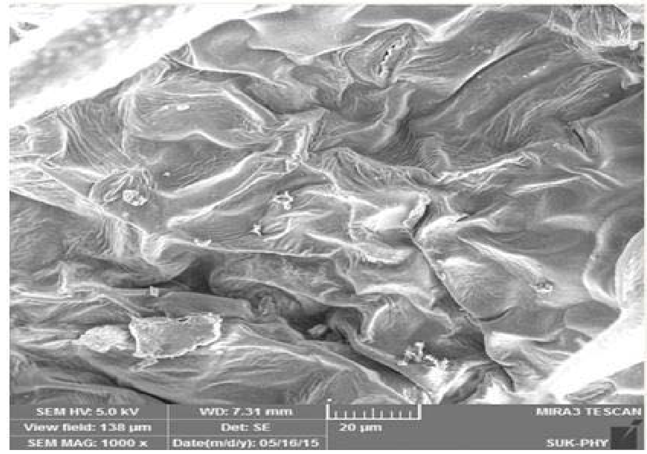


Plate 3 (b): FE-SEM image of sunflower leaf sprayed with 400 ppm nano ZnS at 35 days after sowing. Relatively lesser deposition of nano ZnS particles indicating the absorption into stomatal aperture (x=20μ).

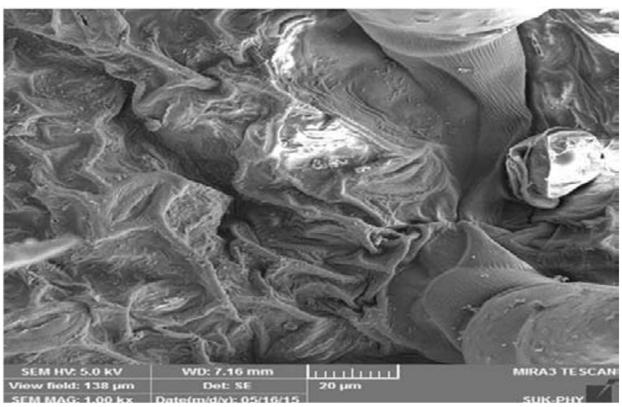


Plate 3 (c): FE-SEM image of sunflower leaf sprayed with 500 ppm nano ZnS at 35 days after sowing. No or lesser deposition of nano ZnS particles indicating the absorption into stomatal aperture (x=20μ).

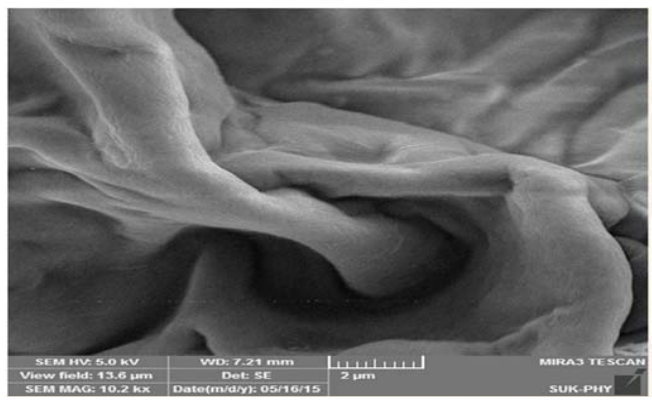


Plate 3 (d): FE-SEM image of sunflower leaf sprayed with water at 35 days after sowing. (x=two μ).

Adopted from Meena (2015)

b) *Above-ground growth*

Foliar application of nano ZnS @ 400 ppm +boron @ 0.5 % recorded highest plant height (146.47 cm), number of green leaves (14.67) which were on par with nano ZnO @ 1,000 ppm + boron @ 0.5 % (Table 2). More availability of nutrient to the plants during initial growth period would have enhanced the growth on account of enhanced photosynthesis. Similar results as that of Zheng *et al* [18] was noticed with nano-TiO_2 treated spinach seeds that resulted in 73% more dry weight, three times higher photosynthetic rate and 45% increase in chlorophyll formation compared to the control over germination period of 30 days. Foliar application of nano ZnS @ 400 ppm +boron @ 0.5 % recorded highest leaf area (555.62 cm^2 $plant^{-1}$), leaf area index (0.377) which were on par with nano ZnO @ 1,000 ppm + boron @ 0.5 % (Table 2). The per cent increases over control were in the range 12-14 % for plant height; 20-26% for number of leaves; 35-36% for leaf area and 38-42% for total dry matter production. These results are in accordance with the findings of Meena and Kumar [11]. The leaf area and leaf area index are major indicators for determining the solar radiation interception, canopy photosynthesis which ultimately results growth and development rate of the crop. The results are in accordance with the findings of Hadiat and Salman [19] who reported that the shoot length, root length, leaf area, chlorophyll, carbohydrate and protein contents of the maize were increased significantly with increase in the concentration of silver nanoparticles from 20 to 60 ppm over control.

Significantly a higher total dry matter production (19.91 g $plant^{-1}$) was recorded with foliar applied nano ZnS @ 400 ppm +boron @ 0.5 % which was on par with nano ZnO @ 1,000 ppm + boron @ 0.5 % (19.41 g $plant^{-1}$). Both the treatments significantly increased the dry matter production to an extent of 40 % over control. However, foliar application of nano Zn particles individually; and conventional $ZnSO_4$ in combination with boron resulted in dry matter production in the range 11.7 - 29.4%. Thus the present study revealed that foliar application of the individual nano micronutrient formulation in combination with boron showed significant effect on growth and yield attributes over sole application of nano formulation.

Crop response to foliar application of nano ZnS and ZnO generally depends on the initial sulphur and zinc status of the soil. Hence, in the present investigation, the response to foliar sprayed nano ZnS and ZnO was attributed to lower soil sulphur and zinc level. The response of sunflower crop to nano ZnS and ZnO application might be due to more synthesis of chlorophyll leading to higher leaf area due to more availability of sulphur [20, 7].

Yield attributes and yield also depends on growth attributes *viz.,* plant height, leaf area, leaf area index and dry matter production per plant. However, significantly a lower dry matter was produced (13.67 g plant^{-1}) with control. Results of present investigation are in conformity with the findings of Raliya and Tarafdar [21] who observed that ZnO NPs induced a significant improvement in cluster bean for plant biomass, shoot and root growth, root area and chlorophyll.

Table 2: Above ground growth parameters of sunflower as influenced by foliar application of green nano zinc particles

Treatment	Plant height (cm)	No. of green leaves	Leaf area (cm^2 plant^{-1})	Leaf Area Index	TDMP (g plant^{-1})
T_1 – Nano ZnS @ 400 ppm	142.73	12.33	533.59	0.361	17.58
T_2 – Nano ZnS @ 400 ppm + boron @ 0.5 %	146.47	14.67	555.62	0.377	19.91
T_3 – Nano ZnO @ 1,000 ppm	135.60	12.67	507.20	0.343	16.48
T_4 – Nano ZnO @ 1,000 ppm + boron @ 0.5 %	144.68	14.00	552.84	0.374	19.41
T_5 – $ZnSO_4$ @ 5,000 ppm	132.7	12.00	432.98	0.316	14.49
T_6 – $ZnSO_4$ @ 5,000 ppm + boron @ 0.5 %	134.03	12.33	463.91	0.333	14.58
T_7 – Water Spray (control)	129.00	11.67	409.72	0.300	14.06
S.Em. ±	0.44	0.44	2.05	0.001	0.14
C.D. at (p = 0.01)	1.83	1.84	8.64	0.006	0.59

c) Below ground growth

Our results demonstrated a significantly higher root growth due to foliar application of nano zinc formulations (Table 3). Root growth parameters enhanced due to foliar application nano ZnS @ 400 ppm +boron @ 0.5 % in root length (73%), root diameter (31%), root volume (87%), surface area (30%) and number of forks (93%) which were on par with application of nano ZnO @ 1,000 ppm + boron @ 0.5 % for root length (71%), root diameter (27%), root volume (70%), surface area (27%) and number of forks (82%) over the control. Our results are in agreement with findings of earlier researchers who reported that ZnO nanoparticles induced a significant improvement in root traits in cluster bean [21] and in tomato [8]; and in sunflower for ZnS nanoparticles [11].

Table 3: Root growth of sunflower as influenced by foliar application of green nano zinc particles

Treatment	Root length (cm)	Average diameter (mm)	Root volume (cm^3)	Surface area (cm^2)	No. of Forks
T_1 – Nano ZnS @ 400 ppm	364.84	0.60	1.06	63.25	2,878.56
T_2 – Nano ZnS @ 400 ppm + boron @ 0.5 %	382.59	0.68	1.25	66.29	3,395.42
T_3 – Nano ZnO @ 1,000 ppm	335.73	0.56	0.96	62.30	2,626.14
T_4 – Nano ZnO @ 1,000 ppm + boron @ 0.5 %	377.80	0.66	1.14	64.75	3,203.94
T_5 – $ZnSO_4$ @ 5,000 ppm	224.67	0.52	0.78	52.28	1,950.02
T_6 – $ZnSO_4$ @ 5,000 ppm + boron @ 0.5 %	295.66	0.54	0.84	56.88	2,123.31
T_7 – Water Spray (control)	221.11	0.52	0.67	51.18	1,756.70
S.Em. ±	4.41	0.007	0.03	0.48	48.712
CD (p = 0.01)	18.57	0.03	0.14	2.02	205.07

d) Yield and yield attributes

Significant response of sunflower to combined application of nano zinc nutrients with boron over conventional source was seen on the seed yield (Table 4). Application of nano ZnS @ 400 ppm + boron @ 0.5% increased the seed yield from 7.06 g to 10.24 g plant^{-1} resulting into 45 % increase; and was on par with nano ZnO @ 1,000 ppm + boron @ 0.5 % (9.88 g plant^{-1}). This might be due partly to more availability of soluble forms of sulphur and zinc in ZnS nano-formulation. The results our earlier findings in sunflower[11] also demonstrated that application of nano ZnS @ 500 ppm at 55 DAS resulted in higher seed yield (5.27 g plant^{-1}) over rest of the treatments; and was on par with 400 ppm nano ZnS sprayed at 35 DAS (4.87 g plant^{-1}).

The average increase in seed yield owing to application of nano ZnS @ 400 ppm +boron @ 0.5 % and nano ZnO @ 1,000 ppm + boron @ 0.5 % over control were 45% and 40%, respectively. These increases in seed yield corroborate with the reports of workers. Meena [12] obtained higher seed yield of sunflower with foliar application of nano ZnS @ 400 and 500 ppm. Prasad *et al.* [7] observed that foliar application of nano ZnO at 15 times lower dose recorded 29.5 % and 26.3 % higher pod yield of groundnut when compared to the chelated $ZnSO_4$.

Foliar application of nano zinc sulphide with 400 ppm at 35 and 55 DAS + boron @ 0.5 % at 40 DAS also recorded significantly higher yield attributes *viz.*, head diameter (10.52 cm), head weight (15.96 g), stalk yield per plant (15.38 g), 100-seed weight (4.36 g), seed filling percentage (81.53) and harvest index (39.97 %) which were on par with nano zinc oxide with 1,000 ppm at 35 and 55 DAS + boron @ 0.5 %. The increments over control were in the range 4-45% for the yield attributes being higher for seed yield (45%); head diameter (37%) and stalk yield (33%). Nadi *et al.* [22] reported similar results wherein foliar application of nano-iron at 6 g l^{-1} during flowering stage increased grain yield faba bean.

Table 4: Yield and yield attributes of sunflower as influenced by foliar application of green nano zinc particles.

Treatment	Head diameter (cm)	Head weight plant^{-1} (g)	Seed yield plant^{-1} (g)	Stalk yield plant^{-1} (g)	Harvest index (%)	100 seed weight (g)	Seed filling percentage
T_1 – Nano ZnS @ 400 ppm	9.14	14.22	8.78	14.69	37.40	4.02	80.43
T_2 – Nano ZnS @ 400 ppm + boron @ 0.5 %	10.52	15.96	10.24	15.38	39.97	4.36	81.53
T_3 – Nano ZnO @ 1,000 ppm	9.63	14.97	8.59	14.21	37.68	4.08	79.38
T_4 – Nano ZnO @ 1,000 ppm + boron @ 0.5 %	10.06	15.90	9.88	15.07	39.59	4.26	80.11
T_5 – $ZnSO_4$ @ 5,000 ppm	8.32	14.00	7.33	12.22	37.51	3.90	78.37
T_6 – $ZnSO_4$ @ 5,000 ppm + boron @ 0.5 %	9.44	13.45	7.41	12.63	37.04	3.81	79.74
T_7 – Water Spray (control)	7.66	13.16	7.06	11.54	37.96	3.67	78.67
S.Em. ±	0.10	0.06	0.09	0.14	0.50	0.03	0.190
C.D. at (p = 0.01)	0.46	0.27	0.42	0.59	2.11	0.11	0.80

e) *Seed oil content and oil yield*

The per cent seed oil content in control increased from 37.06 to 41.15 with the foliar application of nano ZnS @ 400 ppm + boron @ 0.5 %. This treatment also significantly increased oil yield (4.21 g plant^{-1}) compared to rest of the treatments to an extent of 59 and 42 % over control and $ZnSO_4$ @ 5,000 ppm, respectively. Further, foliar application of ZnO @ 1,000 ppm alone and in combination with boron @ 5 % also increased the seed oil content and oil yield to an extent of 23 and 42 per cent, respectively (Table 5). This increase in oil content is attributed to efficient fatty acid synthesis wherein, acetyl Co-A is converted into malonyl Co-A. This conversion is mediated by enzyme thiokinase, the activity of which depends on sulphur supply. Moreover, acetyl Co-A itself contains sulphur and sulphur hydroxyl group [23]. Hence the sulphur containing nano ZnS formulation might have accelerated this process. However, in depth investigation is needed to ascertain the exact mechanism.

Our finding that the percentage of oil in achenes is increased by application of beneficial nanoparticles corroborates these earlier results. Zareii *et al*. [24] found that foliar application of iron nanoparticles during the reproductive growth stage significantly improved the oil percentage in safflower. Because of their very small size, these particles offer a large contact surface per unit of mass and can pass through different protective barriers. Undoubtedly, nanoparticles compared to bulky particles interact better with intracellular processes, and this can partially explain their greater effectiveness. The present findings are consistent with the results of other research indicating that exogenous application of nanoparticles can significantly improve plant growth [8, 25, and 26].

Table 5: Oil content, oil yield, sulphur uptake and zinc uptake in sunflower as influenced by foliar application of green nano zinc particles

Treatment	Seed oil content (%)	Oil yield (g plant^{-1})	S uptake (mg plant^{-1})	Zn uptake (mg plant^{-1})
T_1 – Nano ZnS @ 400 ppm	41.07	3.60	53.80	0.36
T_2 – Nano ZnS @ 400 ppm + boron @ 0.5 %	41.15	4.21	54.40	0.39
T_3 – Nano ZnO @ 1,000 ppm	37.95	3.26	37.00	0.35
T_4 – Nano ZnO @ 1,000 ppm + boron @ 0.5 %	38.11	3.76	37.67	0.38
T_5 – $ZnSO_4$ @ 5,000 ppm	38.83	2.84	47.77	0.28
T_6 – $ZnSO_4$ @ 5,000 ppm + boron @ 0.5 %	38.85	2.88	45.67	0.29
T_7 – Water Spray (control)	37.59	2.65	38.67	0.24
S.Em. ±	0.26	0.040	0.97	0.008
C.D. at (p = 0.01)	1.10	0.17	4.09	0.03

f) Sulphur and Zinc uptake of sunflower

Effect of foliar application of nano Zn particles exerted significant influence on sulphur uptake. Higher sulphur and zinc uptake was noticed in nano ZnS @ 400 ppm + boron @ 0.5 %. This was followed by $ZnSO_4$ treatments for uptake of sulphur and ZnO nanoformulation for uptake of zinc (Table 5). This might be due to higher biomass production leading to higher uptake of nutrients from soil and fertilizers encapsulated in nanoparticles will increase the uptake of nutrients [27]. The uptake rate depends on the size and the surface properties of the nanoparticles. Nanoparticles could enter the xylem via the cortex and the central cylinder and may accumulate in the vacuole. Dietz and Hearth [28] reported that uptake rate depends on the size and the surface properties of nanoparticles.

IV. Conclusions

In the present investigation, the results showed that Zn nanoparticles (<100 nm) could enhance the growth and yield of sunflower plants. The FE-SEM images revealed the complete absorption of nano zinc sulphide and zinc oxide particles by leaves. Zinc, sulphur and boron are important nutrients for oil seed crops like sunflower and found to respond positively to foliar application. The results of the present study suggest that ZnS and ZnO in nano scale form are absorbed by plants to a larger extent compared to bulk $ZnSO_4$. Future *In vivo* studies to determine the toxicity of these NPs are necessary. The growth and yield attributes were increased due to application of nanoformulations of zinc. Thus, it can be concluded that foliar application of zinc sulphide @ 400 ppm at 35 and 55 days after sowing and boron @ 0.5 % at 40 DAS followed by application of nano zinc oxide @ 1,000 ppm at 35 and 55 DAS + boron @ 0.5 % at 40 DAS found optimum for sunflower crop for obtaining higher growth, yield and oil content.

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Conflicts of interest

Authors declare no conflicts of interest.

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