DOI: http://doi.org/10.32792/utq.jceps.10.01.01

Synergistic Effect of ZnO NPs and salicylic acid on Chlorophyll a, b and Carotene content of wheat (*Triticum aestivum L)* leaves

Fatima Hassan sabbar **Faiq Hassan Ali**

Faiq_alradi73@mu.edu.iq

Department of Biology ,Collage of Science, Al-Muthanna University, Iraq

Received 28/5/2023, Accepted 18/6/2023, Published 21/9/2023

Abstract

The aim of this study economic feasibility of using available materials, inexpensive and environmentally friendly as fertilizers for wheat plants .also, Increasing wheat yield by improving the overall vegetative and physiological characteristics. The experiment was for the wheat plant cultivars utilizing two types, *IPA99* and *Abograib*. plants sprayed with three different concentrations of ZnoNPs & SA (0, 50, 75, and100) ppm in three different Treatments,T1 foliar spraying SA , T2 foliar spraying ZnoNPS , and T3 foliar spraying SA & ZnoNP. The results showed the best effect of T3 at75 ppm on the Chlorophyll a, Chlorophyll b and Carotene on *IPA99* cultivar, on the other hand T1 display better effect on Chlorophyll a, Chlorophyll b and Carotene on both cultivars. while ,T3 at 100 ppm show less effects on both cultivars compared to the other treatment.

Key Words: ZnONPs, Salicylic acid , *Triticum aestivum L*, Chlorophyll a,b , Carotene.

Introduction:

Wheat, scientifically named *Triticum aestivum L*., holds the distinguished title of the "King of cereals" and belongs to the Poaceae family. It is renowned as the most extensively cultivated crop worldwide. With over 40% of the global population relying on it as a primary food source, wheat has become a staple in many diets. [1]. Besides its significant agricultural value, wheat is highly beneficial for human health. It serves as an excellent source of essential nutrients like B-vitamins, protein, calcium, and dietary fiber, making it a highly nutritious food [2]. Moreover, wheat contributes essential nutrients, vitamins, amino acids, as well as beneficial phytochemicals and dietary fiber components, which are especially abundant in whole grain products, to promote overall human nutrition [3].

Nanotechnology possesses a diverse array of applications. It involves utilizing particles at the nanoscale to enhance agricultural productivity and address longstanding challenges using unconventional methods. By employing nanostructured formulations, which incorporate mechanisms like targeted delivery, slow/controlled release, and conditional release, active ingredients can be released with greater precision in response to environmental conditions and biological requirements. This capability positions nanotechnology as a promising tool for fostering sustainable agriculture, particularly in developing nations [4].

Zinc (Zn) deficiency poses a significant health concern for both crops and humans, particularly in developing countries where inadequate dietary intake is prevalent. A key contributing factor to this issue was the widespread consumption of Zn-deficient cereals worldwide. To address this, nanotechnology emerges as a potential solution for enhancing the nutritional value of crops, with engineered nanoparticles (NPs) serving as viable fertilizers. Specifically, zinc oxide (ZnO) NPs can be employed for this purpose [5]. Zinc plays a crucial role in supporting plant growth, as it is essential for maintaining a proper balance of all necessary nutrients, resulting in normal growth and optimal yield. It contributes to various beneficial aspects, including enhanced emergence, faster establishment of stands, healthier root growth, increased plant vigor, and improved yield. Furthermore, the deficiency of Zinc triggers the uptake of phosphorus into older leaves to the point of toxicity, while the presence of boron enhances plants that would otherwise have toxic effects in wheat, in cases where Zinc is deficient [6].

Salicylic acid (SA) was a plant hormone with diverse signaling functions in plant growth, development, and responses to environmental stresses [7]. It was a simple phenolic acid with a beta-hydroxy structure that was originally discovered in willow trees, and its name originates from the Latin word "salix" [8]. The phytohormone salicylic acid (SA) is well-known for its role as a signaling molecule in plant defense mechanisms, but its influence on plant growth regulation has not been fully explained until now. Recent research has provided increasing evidence suggesting that SA plays vital roles in the regulation of cell division and cell expansion, which are critical processes determining the overall size of plants [9].

The aim of this study is to use natural , economical inexpensive and environmentally, friendly materials at fertilizers , such as salicylic acid and zinc oxide to improve the growth of wheat plant in general by improving its physiological characteristics .

Material and Methods:

Preparation of ZnO NPs:

To create a stock solution of ZnO nanoparticles (NPs), 1 gram of previously measured ZnO NPs was dissolved in 1 liter of distilled water, resulting in a concentration of 1000 parts per million (ppm). To ensure accurate chemical preparations and minimize errors, the desired concentrations of 0, 50, 75, and 100 ppm were prepared using the titration law ($N1 \times V1 = N2 \times V2$). Separate quantities of ZnO NPs corresponding to each concentration were placed in individual 1-liter flasks for each treatment [10].

Preparation of Salicylic Acid

A stock solution of salicylic acid (SA) was prepared by dissolving 1 gram of SA in a specific volume of distilled water, resulting in a concentration of 1000 parts per million (ppm). To ensure accuracy in chemical preparations, the titration law (N1 × V1 = N2 × V2) was employed to create the desired concentrations of 0, 50, 75, and 100 ppm. Distilled water was used as a solvent and stirred thoroughly. Each treatment required separate quantities of the prepared solutions, which were placed in 1-liter flasks. The experiment followed a factorial design based on a completely randomized design (CRD) with three replicates. In total, there were nine treatments for each wheat cultivar, including a control treatment [11].

T1= (50+75 and100) ppm SA by foliar spraying .

T2= (50+75 and100) ppm ZnO NPs foliar spraying.

T3= (50+75 and100) ppm SA foliar spraying method + ZnO NPs (50+75 and100) foliar spraying.

Plant Cultivation

This experiment was carried out in a natural greenhouse environment at Al Muthanna University, specifically the College of Sciences. Two varieties of plant seeds, *Triticum aestivum L.* (*IPA 99*) and (*Abo-graib*), were used for the experiment. The seeds were planted in pots weighing 5 kg, with each treatment consisting of three replicates. The planting process involved sowing 20 seeds of each variety at a depth of 2 cm on October 11, 2022. Plastic pots with dimensions of 22 cm in diameter, 19.4 cm in height, and a soil capacity of 5 kg were utilized, with a base diameter of 16.3 cm. After 45 days from sowing, the number of seedlings was reduced to eight seedlings per pot. The pots were irrigated to maintain 75 % of the field capacity. When the plants reached an age of approximately 47 days, they were sprayed with a combination of SA and ZnO NPs for three weeks, divided into three separate periods. After 60 days, physiological parameters were measured, and the practical experimental procedures were conducted.

Result and Discussion

Statistical Analysis

The data obtained in the study were analyzed using the Completely Randomized Design (CRD) of treatment, as described by [12]. Significant differences among the means of the treatments were determined by conducting an analysis of variance (ANOVA) with Duncan's new multiple range test at a significance level of $p \leq 0.05$. The statistical analysis was performed using the SAS program.

The synergic effect of ZnO NPs and SA on Chlorophyll a

The results of the table (1) below Show that ZnO PNs and SA resulted in a significant increase in Chlorophyll a of the wheat plant, the highest value was recorded of the, at 75 ppm concentration , to cultivar *(IPA99*) and in the method of synergic ZnO PNs + SA (T3) it was (2.285)mg.g^{-1}, The highest values were for *IPA99* in both concentration (50 ppm&75ppm) involving the

T1&T2, while the second value was (2.222) $mg.g^{-1}$ for the ZnO PNs + SA (T3) at 75ppm in *Abograib* . on the other hand highest value of foliar ZnONPs was (2.145) mg.g-1 in *IPA99*, obviously the concentration 100 ppm in the methods and in both cultivars was less than control., by taking the minimum values (1.327 , 1.327) $mg.g^{-1}$ respectively in comparing with control value (1.956,1.873) mg.g-1.

Table (1)The synergic effect of ZnO NPs and SA on Chlorophyll a ($mg.g^{-1}$)

Cultivars	Treatment methods	Con.ppm control	50	75	100	Interference cultivars ×treatment	Effect of cultivars	Effect of treatment
IPA99	T_1	2.026l	2.117 g	2.178d	1.333r	1.873c		
	T_2	1.993 m	2.107h	2.145e	1.333r	1.845d		
	T_3	1.983 n	2.196c	2.285a	1.327r	2.085a		
Abo–graib	T_1	1.976 o	12.056j	2.096i	1.333r	1.836e		
	T_2	1.956 p	2.032k	2.094i	1.327r	1.755f		
	T_3	1.837 q	2.133f	2.222b	1.327r	1.968b		
Interference cultivars × Con.	*IPA99*	1.986 d	2.093b	2.188a	1.587f		1.936a	
	Abo–graib	1.879 e	2.007c	2.090b	1.327g		1.851b	
Interference treatment × Con.	T_1	2.020 g	2.070e	2.146c	1.603j			1.920b
	T_2	1.984 h	2.050f	2.079d	1.330k			1.795c
	T_3	1.711i	2.169b	2.232a	1.330k			1.956a
Effect of Con.		1.940 c	2.038b	2.140a	1.457d			

Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

The synergic effect of ZnO NPs and SA on Chlorophyll b

According to the data presented in Table 2 below, it is evident that the combination of ZnO PNs and SA led to a noteworthy enhancement in the Chlorophyll b levels of wheat plants. The greatest increase was observed in the cultivar named IPA 99 at a concentration of 75 ppm. Particularly, the synergistic effect of ZnO PNs and SA in the T3 treatment group resulted in the highest Chlorophyll b value (1.142) mg.g^{-1}, The highest values were for *IPA 99* in both concentration (50 ppm&75ppm) involving the T1&T2, while the second value was(0.959) mg.g^{-1} for the ZnO PNs + SA (T3) at 75ppm in *Abograib* . On the other hand highest value of foliar ZnONPs was (0.936) mg.g^{-1} in *IPA99*, obviously the concentration 100 ppm in the methods and in both cultivars was less than control ,by taking the minimum values (0.387 , 0.387)mg.g^{-1} respectively in comparing with control value (0.592,0.524) mg.g^{-1}

Table (2)The synergic Effect of ZnO NPs and SA on Chlorophyll b(mg.g^{-1}) .

Cultivars	Treatment methods	Con.ppm				Interference cultivars ×treatment	Effect of cultivars	Effect of treatment
		control	50	75	100			
IPA99	T_1	0.730m	0.867g	0.936d	0.410s	0.707c		
	T_2	0.730n	0.867h	0.936e	0.410s	0.627d		
	T_3	0.729o	0.959c	1.142a	0.387t	0.890a		
Abo–graib	T_1	0.638p	0.753k	0.867i	0.410s	0.627e		
	T_2	0.592q	0.730l	0.798j	0.387t	0.621f		
	T_3	0.524r	0.890f	0.959b	0.387t	0.787b		
Interference cultivars × Con.	*IPA99*	0.722e	0.806b	0.859a	0.654g		0.739a	
	Abo–graib	0.706f	0.767d	0.775c	0.387h		0.680b	
Interference treatment × Con.	T_1	0.764g	0.798e	0.832c	0.558j			0.704b
	T_2	0.684h	0.775f	0.809d	0.398k			0.667c
	T_3	0.683i	0.901b	0.912a	0.398k			0.758a
Effect of Con.		0.756c	0.771b	0.791a	0.520d			

Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

The synergic effect of ZnO NPs and SA on carotene content

The results of the table (3) below show that ZnO PNs & SA resulted in a significant increase in carotene of the wheat plant, the highest value was the height of the, at 75 ppm concentration , to cultivar (*IPA99*)and in the method of synergic ZnO PNs + SA (T3) it was (1.774) $mg.g^{-1}$, The highest values were for *IPA 99* in both concentration (50 ppm&75ppm) involving the T1&T2, while the second value was (1.308) $mg.g^{-1}$ for ZnO PNs + SA (T3) at75ppm in *Abograib* . Furthermore highest value of foliar ZnONPs was (0.906) $mg.g^{-1}$ in *IPA99,* obviously the concentration 100 ppm in the methods and in both cultivars was less than control., by taking the minimum values (0.382, 0.214) $mg.g^{-1}$ respectively in comparing with control value (0.522, 0.470) $mg.g^{-1}$.

Table (3)The synergic Effect of ZnO NPs and SA on carotene contain ($mg.g^{-1}$) .

Cultivars	Treatment methods	Con.ppm				Interference cultivars ×treatment	Effect of cultivars	Effect of treatment
		control	50	75	100			
IPA99	T_1	0.681bcd	0.882bcd	0.930bc	0.404cd	0.691a		
	T_2	0.681bcd	0.882bcd	0.906bcd	0.385cd	0.654a		
	T_3	0.681bcd	1.257ab	1.774a	0.385cd	0.913a		
Abo–graib	T_1	0.661bcd	0.709bcd	0.835bcd	0.393cd	0.650a		
	T_2	0.522cd	0.686bcd	0.759bcd	0.382cd	0.634a		
	T_3	0.470cd	0.882bcd	1.308ab	0.214d	0.875a		
Interference cultivars × Con.	*IPA99*	0.681bc	0.874ab	1.184a	0.561bc		0.814a	
	Abo–graib	0.667bc	0.715bc	0.724bc	0.483c		0.658b	
Interference treatment × Con.	T_1	0.772ab	0.782ab	0.833ab	0.532b			0.781a
	T_2	0.591b	0.782ab	0.821ab	0.428b			0.644a
	T_3	0.548b	1.119a	1.230a	0.398b			0.783a
Effect of Con.		0.698ab	0.717ab	0.926a	0.603b			

Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

These results are consistent with the results of the study [13], and the results for the chlorophyll a ,b of this Investigation were similar to the results of [4] As foliar spraying ZnoNps to increase the amount of chlorophyll in wheat plant. also was consistent with the results of the study[14] As foliar spraying SA.

Zinc (Zn) is an essential micronutrient that plays a crucial role in the optimal growth of wheat plants. Its application has been observed to increase grain yield and dry matter production in wheat [15]. Zn also contributes to enhancing the yield components of wheat, as reported by [16]. Zn performs vital functions in plant metabolism, and its deficiency can have detrimental effects on plant growth. It is a key element for controlling enzymatic systems in plants. ZnO nanoparticles (NPs) are preferred over conventional ZnO fertilizers due to their solubility, accessibility, and responsiveness, which are attributed to their nanoscale size and larger specific surface area [17].Furthermore, plants treated with salicylic acid (SA) exhibit higher chlorophyll content, photosynthetic rates, and increased Rubisco activity [18].

In contrast, the adverse effects of high concentrations of ZnO can be attributed to their toxicity towards nucleic acids and cell division. This observation is supported by previous studies conducted on Arabidopsis thaliana [19], which demonstrated the phytotoxic effects of elevated levels of ZnO nanoparticles (NPs). Furthermore, [20] reported that high concentrations of ZnO NPs resulted in abnormalities during mitotic division in cells.

Conclusion:

The response of wheat plants to the synergistic treatment of each of SA + ZnOPNs more than the rest of the other treatments that are spraying SA alone or spraying ZnOPNs. the physiological features of the plant improved .The positive effect of ZnO NPs &SA on wheat plant by increasing the Chlorophyll a, Chlorophyll b and Carotene contain and thus stimulating the physiological and vegetative characterist

Reference

[1] Giraldo, P. ; Benavente, E. ; Manzano-Agugliaro, F. and Gimenez, E. (2019)."Worldwide research trends on wheat and barley: A bibliometric comparative analysis." Agronomy ., 9.7: 352.

[2] Kumar, P. ; Yadava, R. K. ; Gollen, B. ; Kumar, S. ; Verma, R. K. and Yadav, S. (2011). "Nutritional contents and medicinal properties of wheat: a review." Life Sciences and Medicine Research ., 22.1: 1-10.

[3] Shewry ,P.R. and Sandra J. Hey. (2015)."The contribution of wheat to human diet and health." Food and energy security .,4.3: 178-202.

[4] Adil, M.S. ; Aslam, Z. ; Ahmad, N. ; Younas, T. ... and Elshikh, M. S. (2022). "Zinc oxide nanoparticles improved chlorophyll contents, physical parameters, and wheat yield under salt stress." Frontiers in Plant Science 13.

[5] Munir, T. ; Rizwan, M. ; Kashif, M. ; Shahzad, A. ; Ali, S. ; Amin, N. and Imran, M. (2018). "effect of Zinc oxcid nanoparticles on the growth and Zn uptake in wheat (Triticum aestivum L.) by seed priming method." Digest Journal of Nanomaterials & Biostructures (DJNB) 13.1

[6] Pandey, M. ; Shrestha, J. ; Subedi, S. and Shah, K. K. (2020). "Role of nutrients in wheat: A review." Tropical Agrobiodiversity., 1.1 :18-23.

[7] Arif, Y. ; Sami, F. ; Siddiqui, H. ; Bajguz, A. and Hayat, S. (2020). "Salicylic acid in relation to other phytohormones in plant: A study towards physiology and signal transduction under challenging environment." *Environmental and Experimental Botany* .,175: 104040.

[8] Rivas-San Vicente, M. and Javier, P. (2011). "Salicylic acid beyond defence: its role in plant growth and development." *Journal of experimental botany* 62.10: 3321-3338.

[9] Li, A. ; Xue, S. and Lijing, L. (2022). "Action of salicylic acid on plant growth." Frontiers in Plant Science 13.

[10] Elewa, S. ; Fatthallah, N. A. ; Nessim, M. I. and El-Farargy, A. F. (2020). "Synthesis and characterization of some tetrazoles and their prospective for aerobic micro-fouling mitigation." Arabian Journal of Chemistry ., 13.12: 8750-8757.

[11] Khandan-Mirkohi, A. ; Khalili, H. M. ; Salami, S. A. and Lesani, H. (2017). "Improving effects of mild cold stress and salicylic acid on growth and physiology of periwinkle (Catharanthus roseus Don.)." International Journal of Horticultural Science and Technology., 4.1 67-78

[12] Antar, S. H. (2010). Statistical Analysis in Scientific Research and SAS Program (2010). University of Mosul, Faculty of Agriculture and Forestry", Ibn al-Atheer Publishing House, conductor, Iraq .

[13] Sarkhosh, S. ; Kahrizi, D. ; Darvishi, E. ; Tourang, M. ; Haghighi-Mood, S. ; Vahedi, P. and Ercisli, S. (2022). "Effect of zinc oxide nanoparticles (ZnO-NPs) on seed germination characteristics in two Brassicaceae family species: Camelina sativa and Brassica napus L." Journal of Nanomaterials 2022: 1-15.

[14] Yıldırım, E. and Dursun, A. (2008) ."Effect of foliar salicylic acid applications on plant growth and yield of tomato under greenhouse conditions." International Symposium on Strategies Towards Sustainability of Protected Cultivation in Mild Winter Climate 807.

[15] Ozkutlu, F. ; Bulent, T. and Ismail, C. (2006). "Effect of zinc humate on growth of soybean and wheat in zinc-deficient calcareous soil." Communications in Soil Science and Plant Analysis., 37.15-20: 2769-2778.

[16] Singh, V. ; Rajvir, S.P. and Totawat K. L. (2004). "Effect of phosphorus and zinc nutrition of wheat (Triticum aestivum) in soils of sub-humid southern plains of Rajasthan." Indian Journal of Agronomy., 49.1: 46-48.

[17] Borm, P. ; Klaessig, F. C. ; Landry, T. D. ; Moudgil, B. ; Pauluhn, J. ;Thomas, K. ... and Wood, S. (2006). "Research strategies for safety evaluation of nanomaterials, part V role of dissolution in biological fate and effects of nanoscale particles."Toxicological sciences ., 90.1 :23-32.

[18] Singh, B. and Usha, K. (2003). "Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress." Plant growth regulation .,39 :137-141..

[19] Lee, C. W. ; Mahendra, S. ; Zodrow, K. ; Li, D. ; Tsai, Y. C. ; Braam, J. and Alvarez, P. J. (2010). "Developmental phytotoxicity of metal oxide nanoparticles Arabidopsis thaliana." Environmental Toxicology and Chemistry: An International Journal., 29.3 :669-675.

[20] Raskar, S. V. and Laware ,S. L. (2014). "Effect of zinc oxide nanoparticles on cytology and seed germination in onion." Int J Curr Microbiol App Sci., 3.2: 467-473.